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Editorial

Whole genome sequencing and metagenomics for public health use in Ethiopia

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The idea of this article is not to address all issues in whole genome sequencing (WGS); rather it is to focus on major areas of this growing science that will fit the existing local needs. Suggestions are also given for what purpose is WGS is to be used at the moment even though, this will continue to be updated as the technology continues to evolve at rapid pace. Stress is also made on issues of capacity development in health science research, research institutions and universities to ensure the uptake and sustainability of this cutting edge health research capability for the present and the coming generations.

The genome or genetic material of an organism is made up of DNA. Each organism is composed of four DNA bases (ATCG). Whole genome sequencing is a laboratory procedure that determines the sequence of the bases in the whole genome of an organism whether it is human, animal, microorganism or plant cells is known as WGS.

WGS technique has brought explosive development in medical research in interims of unraveling complex mysteries of life. The technique is comparatively user-friendly that can be of value both in developing and developed countries. Sequencing method was initially developed by Sanger et al. (1977) and over several decades it has gone through substantial changes to result into efficient bench top technology for research and laboratory use (Kwong et al. 2015).

WGS will provide information as never before in detection and characterizing of disease causing agents. Normally, genomic tests which involve human DNA can have substantial ethical ramifications; however tests based on pathogen analysis are free from these. This is because in the laboratory analysis of the genetic test science, it is the pathogen's genetic material in question rather than the patient's DNA which are tested. A traditional culture technique for example, is very slow and labour-intensive. It takes several days and in some cases (in tuberculosis) several weeks and months to identify the cause of an infection and establish which treatments could be effective. WGS could transform the way these laboratories function, helping clinicians to manage patients with suspected infections faster and more effectively. On the other hand, metagenomics refers to both a research technique and research field and the field can be defined as the genomic analysis of microbial DNA in their natural environment, including skin, mouth, and gut samples as well as soil, water and plant samples to understand the effect of microbial communities in health. Metagenomics tools enable the population analysis of un-culturable or previously unknown microbes. This is important as only around 1-2% of bacteria can be cultured in the laboratory (Wade 2002). The ability to identify microbes without a priori knowledge of what a sample contains is opening new doors in disciplines like microbial ecology, virology, microbiology, environmental sciences and biomedical research. The combination of both WGS and metagenomics has become a powerful tool for generating novel hypotheses in human disease (Kwong et al. 2015).

Using WGS strain typing has been improved with far more discriminatory power provided by culture or by other molecular techniques including rRNA gene based analysis. WGS technique is providing increasingly accessible means for tracking disease outbreaks and sequence-based information of drug resistance as well as virulent gene tracing even though some limitations are observed (Taylor et al. 2015).

Currently, WGS cannot be used in medical laboratories for the detection of disease causing organism for routine purposes. Given the need and the standard of local capacity WGS can have increasing use in Central and Regional/Medical School Laboratories for the purpose of epidemiological and related studies. This approach will initiate the capacity development of laboratory techniques of WGS in institutions together with enhanced manpower development in bioinformatics (Gupta et al. 2014) that can handle the huge amount of sequence data that is generated at national level. Using exiting automated technology of WGS huge sequence data can be generated, however, such large data will not be helpful for public health unless properly analyzed and interpreted by bioinformatics scientists and related experts. Therefore adequate

Sciences training in bioinformatics both in number and quality is very much essential.

Given the potential of WGS in the control of infectious diseases (and non-infectious diseases for that matter) the wider application of WGS in Ethiopia is very much within sight if we invest on existing and upcoming young researchers to exploit their avant-garde stamina for the good of the country in earnest.

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Safety and nutritional quality of commonly consumed commercial edible oils in Addis Ababa

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Abstract
Background: Keeping food safety and quality are the major components of food security and protecting human health. Directly or indirectly, edible oils are involved in every day dish; and due to this, long time preference on the type of edible oil should pay off to the health and development of individuals.
Objective: To analyze physicochemical quality and safety of commonly consumed commercially available oils in Addis Ababa.
Methods: Sixteen different types of solid and liquid edible oils were collected from main shops and supermarkets in Addis Ababa from October to December, 2015. Following the sample collection, physicochemical properties were analyzed using standard methods.
Results: The solid oils have significantly higher iodine value, saturated, monounsaturated, Poly unsaturated fatty acids, Cis, Trans, Omega-6 fats ($p<0.05$) as compared with the liquid. Palm oils have significantly higher ($p<0.05$) trans, saturated, monounsaturated and lower omega-6 and cis fats.
Conclusion: Imported palm oils contain surplus amount of saturated fatty acid; and according to the consumption, it is usually surpass the WHO daily recommendation amount. Consequently, to avoid the long term health effect, substituting liquid oils in place of solid is recommended for their many health benefits. Locally produced edible oils contain good Poly unsaturated fatty acids, omega fats, vitamin A and vitamin E; however they need to pass through all the necessary refinery processes such as degumming, deacidification, bleaching, filtration and deodorization steps.
Key words: Fatty acids, cardiovascular disease, and physicochemical properties.

Introduction
Oil and fat are important constituents of our diet and contribute significantly for the development and regulation of different functions of our body (Tahir et al. 2012). Vegetable oils have been recognized as sources of essential fatty acids, carrier of fat soluble vitamins and enhance palatability of the foods we eat by providing texture, mouth feel, impartation of flavors and contribution to the feeling of satiety after eating (Otunola et al. 2009). However, some vegetable oils are not up to standards to meet the quality, safety, consumer satisfaction in terms of their physicochemical properties, texture and stability of the food products (American Dietetic Association 2007).

Industrial processing, especially catalytic hydrogenation of vegetable oils, affects their fatty acid composition. Higher hydrogenation of oil is directly proportional to higher saturation, solidification and presence of trans fatty acid. Highly saturated fatty acid diets have been found to increase the level of cholesterol (Fattore et al. 2014). Several studies have shown that palm oil has a hypercholesterolemic effect because of its high saturation to 50% palmitic acid (C16:0). This high source of saturated fats in the human diet thought to have detrimental effect on human health, particularly when compared with polyunsaturated fatty acids (PUFAs) (Bautista et al. 2001).

According to the WHO report, there is convincing evidence that palmitic oil consumption contributes to an increased risk of developing cardiovascular diseases (CVDs). These evidences show that dietary saturated fatty acids (SFAs) increase blood total cholesterol (TC) and low density lipoprotein (LDL) cholesterol, which are known to be risk factors for coronary heart disease (CHD) and cardiovascular disease (CVD) (Ness 2004). WHO recommended that; to improve the diet in both populations and individuals level; one of the strategy focused is diet and physical activity (DPAS); limiting energy intake gain from total fats and shift fat consumption from saturated to unsaturated fats and toward the elimination of TFA (Nishida and Uauy 2009).

The liquid vegetable oils produced in Ethiopia have higher acidity than the country codex Standard (Atnafu and Bedemo 2011). According to data from food, beverage and pharmaceutical industry development institute (April, 2017) In Ethiopia imported and locally processed edible oils are used widely; however the locally processed edible oil production is very low, contributing (1.57%) of the country's supply; for this reason currently the

imported palm oils sharing the biggest contribution (96.73%) of the supply in the country (unpublished data). The objective of this research is to analyze the quality, safety and nutritional content of edible oils which are commonly sold in Addis Ababa.

Materials and Methods

Physicochemical analysis of oils: The study was conducted in two phases. The first phase was assessing the physicochemical and fatty acid profile of the oils. And this study serves us a base line for continuing animal trial, which is not included in this paper. A cross sectional study of 16 different edible oils were selected and collected which were found in main shops and supermarkets of Addis Ababa from October to December, 2015. Most of edible oil brands of solid and liquid oils which were stored under room temperature in the market were included. Following sample collection, physicochemical properties of the oils were analyzed.

Physical properties of the edible oils includes: color, odor, whether refined, labeling of the expiry date, present with settle able solids, liquidity of the oil and fortified or not. Chemical properties of edible oils includes: Total fat, peroxide, iodine, acid value, refractive index and specific gravity of the oils were determined using AOAC standard method, (Firestone and Yarawecz 2016); fatty acid profiles were analyzed using Christopherson and Glass (1969); and sterol was analyzed using dole extraction technique as described by Smuts et al. (1992).Vitamin A (Thomas et al. 2011) and Vitamin E (Manz and Philipp 1981) levels were also determined by using high performance liquid chromatography.

Statistical analysis: Data of the physical properties and nutrient contents of the oils were analysed using SPSS statistical software version 20. Descriptive statistics was used to summarize the findings, and Student t-test and ANOVA were respectively used to test any significant difference between solid and liquid oils, and among locally produced and imported edible oils. This study was approved by IRB with the given approval reference No. of EPHI 6.13/821 in 2015.

Results and discussion

Physical characteristics of the oils: Among the total of 16 analyzed oil brands, seven were produced in Ethiopia. They haven't fulfilled the necessary food labeling requirements such as nutrition facts, fortification information, expiry date, cholesterol level and refinery information.

Five of the locally produced oils were not refined, due to this they colored deep yellow or yellow brown and three of them were found with foreign residual matter. The rest nine brands were imported oils from different countries and five of them were fulfilled necessary oil product labeling requirements however the other four still need improvement in their labeling. All the solid oils included in this study were imported oils, in fact no solid form oil produced in Ethiopia so far (Table1).

Table 1: Physical properties of commercially available edible oils in Addis Ababa

Code	Color	Refining process	Labeling quality	Local/Imported	Expiry date	Existed State	Foreign residue	Fortified
Hamar	Light-yellow	Not refined	Poor	local	Have not	liquid	Have not	No
OO-loret	Greenish-yellow	Not refined	Poor	Imported	Have	liquid	Have not	No
SBO-V	Light-yellow	Refined	Met	Imported	Have	liquid	Have not	No
SBO-GS	Light-yellow	Not refined	Poor	Imported	Have	liquid	Have not	No
CO-Ver	Orange-Yellow	Refined	Met	Imported	Have	liquid	Have not	No
SFO-Hat	Light-yellow	Refined	Met	Imported	Have	liquid	Have not	No
SFO-Moj	Light-yellow	Refined	Poor	local	Have not	liquid	Have not	No
Ni-T&M	Deep-yellow	Not refined	Poor	local	Have	liquid	Have	No
Ni-Dan	Deep-yellow	Not refined	Poor	local	Have	liquid	Have not	No
Ni-NL	Greenish-yellow	Not refined	Poor	local	Have not	liquid	Have	No
CSO-Moj	Yellow- brown	Not refined	Poor	local	Have not	liquid	Have	Yes
SSO-NL	Deep yellow	Not refined	Poor	local	Have not	Liquid	Have	No
Pal-A	light yellow	Refined	Met	Imported	Have	solid	Have not	No
Pal-O	light yellow	Have not	Poor	Imported	Have	solid	Have not	Yes
Pal-V	light yellow	Refined	Met	Imported	Have	solid	Have not	No
SL-Yem	light yellow	Refined	Poor	Imported	Have not	solid	Have not	Yes

Note: Oil brands were presented as codes so as to adhere with trade practice and consumers' protection rights.

Chemical analysis of the oils: Of the 16 analyzed oil samples, acid value of the six locally produced and one imported was out of the country edible oil standard (>0.6 mgKOH/g) (Table 2). Being hydrogenated or solid was significantly associated with high Trans(Craig-Schmidt 2006), saturated and MUFA but with low Cis, PUFA, omega-3, and omega-6 fatty acids, sterol level, peroxide and iodine value (Table 3). Locally produced oils had higher acid value (1.78 mg KOH/g) and peroxide value (4.42 meq O_2/kg) than the imported (0.52 mgKOH/g and 3.59 meqO_2/kg respectively), which attributed to incomplete refinery process, presence of moisture which results hydrolysis and elevated temperature (Vidrih et al. 2010).

Table 2: Chemical compositions of edible oils in Addis Ababa

Code	Peroxide value (meq O_2/kg)	Acid value (mgKOH/g)	Iodine value (gI_2/100g)	Ref. index at 40 °C	Sp. gravity at 20 °C
Liquid oils					
Hamar	2.07	1.01	133.72	1.477	0.921
OO-loret	6.0	0.60	70.00	1.47	0.915
SBO-V	3.02	0.60	128.85	1.476	0.920
SBO-GS	1.3	0.40	132.44	1.476	0.920
CO-Ver	3.25	0.60	119.60	1.475	0.920
SFO-Hat	4.39	0.40	126.56	1.475	0.920
SFO-Moj	8.81	0.71	120.00	1.475	0.921
Ni-T & M	5.14	1.11	122.76	1.476	0.921
Ni-Dan	5.88	1.86	124.77	1.476	0.921
Ni-NL	3.32	4.13	116.15	1.476	0.890
CSO-Moj	3.9	0.40	105.96	1.474	0.925
FSO-NL	1.82	3.22	145.94	1.478	0.923
Mean	4.08 ± 2.13	1.25 ± 1.22	120.56 ± 18.77	1.475 ± 0.0	0.92 ± 0.0
Median	3.61	0.66	123.77	1.476	0.920
Solid oils					
Pal-A	1.23	0.50	57.52	1.467	0.908
Pal-O	1.34	0.40	62.33	1.467	0.910
Pal-V	4.0	0.40	55.30	1.467	0.910
SL-Yem	0.95	0.80	49.23	1.468	0.910
Mean	1.88 ± 1.42	0.53 ± 0.19	56.10 ± 5.44	1.467 ± 0.0	0.910 ± 0.0
Median	1.29	0.45	56.41	1.467	0.910

Note: The normal ranges for acid value in refined edible oils by Codex in 1993is 0.6 mg KOH/g of oil; accordingly six of the seven locally produced edible oils were unfit to the standard.

The high acidity result in the local unrefined edible oils was agree with the study done by Rajko et al. (2010), which the result shows that unrefined edible oils have high acidity than refined oils. And also refinery process increase the shelf life by removing moisture, acylglycerols, phospholipids, free fatty acids, metal ions and metal complexes which act as a pro-oxidant increasing the oxidation of acylglycerols (Vaisali, et al. 2015). Because of higher degree of unsaturation, liquid oils had higher mean iodine value (120.6±18.8g/100g) than solid oils (56.1±5.4g/100g); nearly 47g per 100g of the solid palm oils were containing saturated fatty acids. Unsaturation feature of liquid oils which make them susceptible to continuous lipid oxidation in air, sunlight or limited moisture also favors to have higher peroxide value (4.08±2.13) than solid oils (1.88 ±1.42). Locally produced liquid oils had slightly higher peroxide value (4.42 meq O_2/kg) than imported oils (3.59meq O_2/kg) attributed to the presence of impurities.

In this study, there was 47% SFAs and 11.57g PUFAs from100g the palm oil (Figure 2) and according to National Food Consumption Survey (EPHI 2013), in Addis Ababa there was edible oil consumption of 27.8g/day/person; 12.80g (115.1 calorie) SFAs and 3.22g (4.83% of the total fat calorie) PUFAs (EPHI 2013) which was higher than WHO`s recommendation to be less than 10% and 6-10% of the total fat calorie for SAFs and PUFAs since their excess level are risks to CVD by raising blood cholesterol and LDL levels. Moreover, several studies showed the correlations between high intakes of industrially produced TFAs and increased risk of CHD (Stender et al. 2006; Tarrago-Trani et al. 2006; Willett 2006).

Table 3: Chemical profile of edible oils used in Addis Ababa

Type of oil	Sterol	Fat	Vit. A	Vit E	SFA	MUFA	PUFA	TFA	CFA	ω3FA	ω6 FA	ω9 FA	ω6/ ω3	EPA	DHA
Hamar	199	99.57	0.06	0	17.31	5.91	76.36	0.20	80.51	1.38	75	7.9	54.3	1.10	0
OO-loret	103	99.69	0.04	2.7	17.2	76.2	6.3	0.01	80.15	0.8	5.5	74.8	6.9	0	0
SBO-vik	112	99.54	0.03	2.25	16.7	21.5	61.3	0.05	74	8	53.3	21.2	6.7	0	0
BO-GS	121	99.66	0.03	9.71	16.1	20.5	63	0.05	73.9	8.2	54.8	20.2	6.7	0.02	0
CO-Vera	319	99.63	0.03	2.32	14.3	30.2	55.1	0.03	83.8	0.82	54.3	29.7	66.2	0	0
SFO-Hatun	155	99.6	0.04	0.05	11.8	27.4	60.4	0.02	86.93	0.32	60.1	27.09	187.8	0.01	0
SFO-Mojo	168	99.41	0.06	0.66	12.95	34.6	51.8	0.05	76.76	2.12	49.4	32.91	23.3	0	0
Ni T & M	162	99.77	0.09	29.49	24.5	41.75	22.4	11.08	40.85	11.42	11	51.97	1.0	1.20	0
Ni-Dana	194	99.78	0.05	0	15.8	23.1	60.9	0.03	78.34	5.16	55.69	22.76	10.8	0.48	0.02
Ni-un	202	99.62	0.05	0	16.8	31.1	51.75	0.01	78.56	3.55	48.2	30.44	13.6	0	0
CSO-Mojo	231	99.88	0.04	0.58	27.36	18.16	54.32	0.05	70.63	0.84	53.52	17.42	63.7	0.42	0.02
FSO-un	136	99.37	0.06	3.2	16.69	21.9	60.72	0.06	51.18	30.66	30.06	21.56	1.0	0.10	0.02
Mean liquid oils	175	99.63	0.05	4.25	17.29	29.36	52.03	1.04	72.98	6.10	45.90	29.64	7.5	0.28	0.00
Sd	60	0.15	0.02	8.40	4.43	17.30	19.08	3.33	13.51	8.54	20.28	17.97	2.4	0.45	0.01
Median	165	99.62	0.04	1.45	16.69	25.25	57.75	0.05	77.55	2.83	53.41	24.9	18.9	0.01	0
Pal- ave	36	99.73	0.04	1.91	45.28	42.68	11.67	0.09	53.52	0.16	11.25	42.43	70.3	0	0
Pal- ole	0	99.8	0.04	22.33	46.22	41.77	11.74	0.08	52.07	0.74	11.02	41.47	14.9	0	0
Pal-vik	37	99.72	0.03	17.89	46.67	41.61	11.3	0.15	52.29	0.23	11.09	41.41	48.2	0.05	0
SL-16	0	99.23	0.07	5.95	50.6	38.1	10.4	0.2	46.5	1.3	9.1	20.2	7.0	1.13	0.01
Mean solid oils	18.25	99.62	0.05	12.02	47.19	41.04	11.28	0.13	51.09	0.61	10.61	40.80	17.4	0.30	0.00
Sd	21.08	0.26	0.02	9.66	2.34	2.01	0.62	0.05	3.13	0.53	1.01	74.8	1.9	0.56	0.01
Median	18	99.75	0.04	11.92	46.44	41.69	11.48	0.12	52.18	0.48	11.05	21.2	23.0	0.03	0

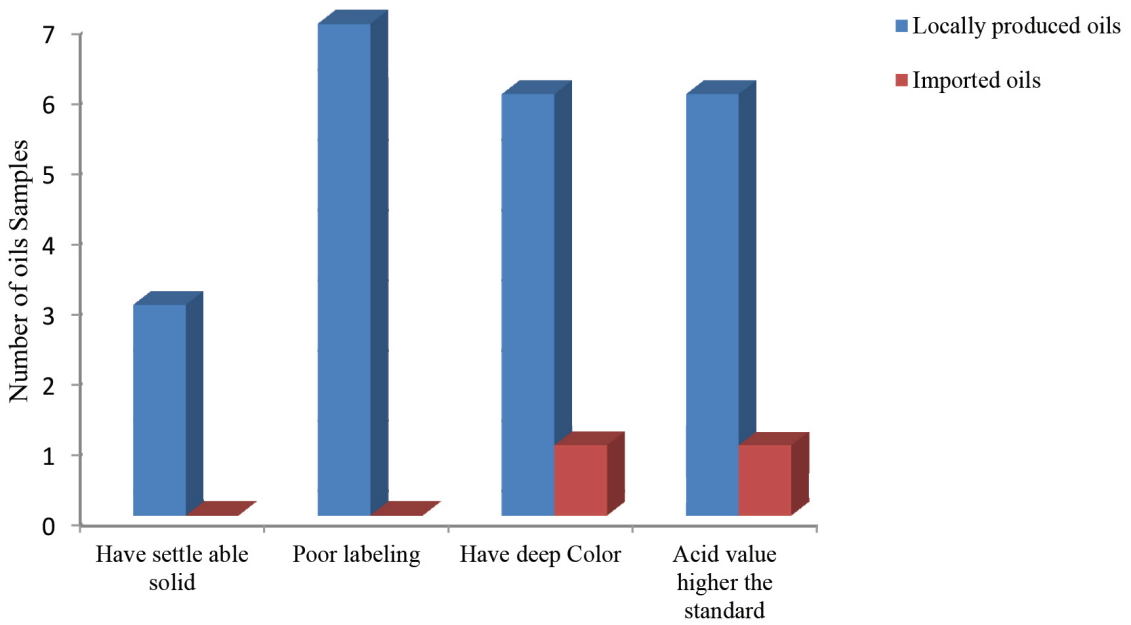

Figure 1: Physicochemical properties of imported and locally produced edible oils

When TFA intake is lowered by 2% (5 g/day), there is a >20% reduction in CHD incidence (Katan 2006; Moss, 2006); foods that contain more than 4g/100g SFAs and TFAs together should not be claimed to be healthy food (Sebastjan et al. 2010). It is recommended to limit TFA consumption less than 1% (approximately 2 g on a 2,000-calorie diet), and saturated fat consumption less than (7%) of the total daily calories (Otunola et al. 2009). Unlike wise, the TFAs constituent of the palm oil in the present study was laid in the acceptable range of daily tolerable limits.

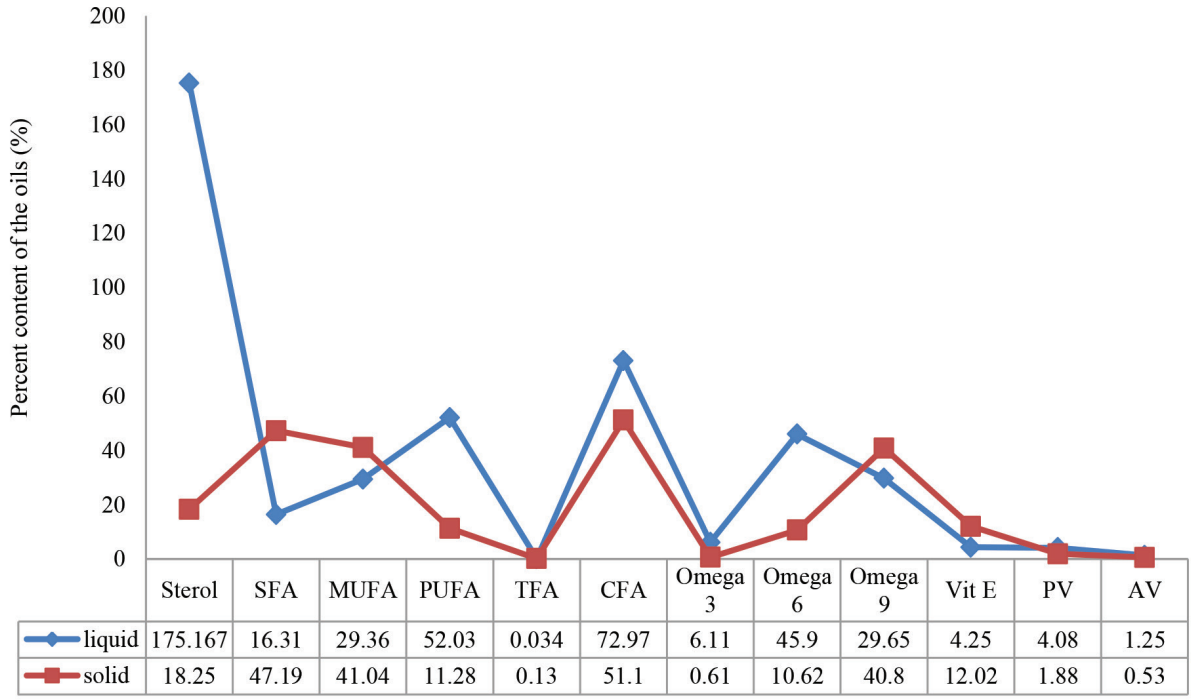

Figure 2: Chemical profiles of solid and liquid oils

Conclusion

Imported oils included in this study were good in labeling; which composed of full information about the product, and they were refined well, clear in appearance and fulfilled oil standard. On other hand, locally produced liquid oils had equivalent quality as imported oils in having sufficient PUFAs content with low saturated FAs, good Omega-6 to Omega-3 ratios, Vitamin A and vitamin E level. However, they did not fulfill labeling requirements and also they need to pass through all the necessary refinery process in the factory; including deacidification, bleaching, refining and deodorization to remove bad odor, unwanted impurities, enhance consumer acceptability and oil shelf life. Palm oils had high Trans, saturated, MUFA and Omega-9 fatty acids with low Omega-3, Omega-6 and cis fatty acids as compared to the liquid vegetable oils. Therefore, it is strongly recommended to use liquid (higher PUFAs) oils in place of solid oils for their many health benefits. As Ethiopia is one of a country producing large amount of oil seeds, perhaps production level of edible oil has to be increased equally to balance the huge demand.

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Incidence of tuberculosis and associated factors among HIV infected people who enrolled in the pre-ART and ART clinics of East Gojjam, North West Ethiopia

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Abstract

Background: The interaction between tuberculosis and HIV infection is complex. These diseases present formidable challenges alone and together as well as pose a major threat to the international community's effort to achieve the health-related United Nations MDG for these infections. However, studies to understand the perspective of tuberculosis incidence among HIV infected people who enrolled in the chronic care units have been little in Ethiopia.

Objective: To determine incidence of tuberculosis and associated factors among chronic HIV care attendants.

Methods: A clinical-based retrospective cohort study was conducted from June to October 2012 in East Gojjam zone, Northern Ethiopia. All adults aged ≥ 15years and enrolled in the chronic care units were included in the study. Data were retrieved by using a pre-tested and structured questionnaire; then entered and cleaned using EPI info version 3.5, 2008 statistical software. SPSS window version 16.0 was also employed for descriptive and logistics regression analysis.

Results: From a total of 964 study subjects, 39 of 482(8.1%) from the pre-ART group and 21 of 482(4.4%) from the ART group developed tuberculosis disease. Being widowed [AOR: 4.02; 95 % CI (1.22, 13.23)], low level hematocrit [AOR: 12.36; 95% CI (4.75, 32.19)], unable to take Cotrimexazole prophylaxis therapy[AOR: 5.89; 95% CI (2.37; 14.66)], below 350 cells/ml CD4 count [AOR:7.7; 95% CI (2.37; 24.93)] from the pre-ART group and being widowed [AOR: 6.68; 95 % CI (1.07; 41.66)], low level hematocrit [AOR: 5.93; 95% CI (1.45, 23.61)], unable to take Cotrimexazole prophylaxis therapy[AOR: 0.071; 95% CI (0.021, 0.242)], below 350cells/ml CD4 count [AOR: 10.05; 95% CI (1.26, 80.14)] from the ART group were some of the factors that had significant association with the incidence of tuberculosis.

Conclusions: In this study, the incidence of tuberculosis was high among pre-ART group than the ART group. Functional status, marital status, baseline hematocrit, CD4count, Cotrimexazole prophylaxis therapy and WHO clinical stage had association with the incidence of TB. Therefore, initiation of ART regardless of CD4 count, viral load or using WHO clinical stage of HIV/AIDS plays a significant role in preventing the co-infection of tuberculosis.

Keywords: HIV, tuberculosis, incidence, pre-ART, ART

Introduction

The interaction between tuberculosis (TB) and human immunodeficiency virus (HIV) infection is complex (Daniel et al. 2008). HIV infection weakens the immune system and increases the likelihood of reactivation, reinfection and progression of latent TB infection to active disease (Sheela et al. 2010; Reuben et al. 2010). TB is the major opportunistic infection (OI) and leading cause of death among people with HIV (Gysje et al. 2010). Each disease presents formidable challenges alone and together (William et al. 2010) and their intricate linkage, poses a major threat to the international community (Haileyesus et al. 2010; Tajeldin and Abdellah 2010).

Magnitude of Tuberculosis and HIV: Since the initial description of HIV as the causative agent of AIDS, more than 60 million people have been infected with the virus, and more than 25 million people have died, globally (Macro et al. 2009). *Mycobacterium tuberculosis* infects 2 billion persons worldwide with 10 million new cases each year. Ninety percent of infected persons never develop clinical disease TB (WHO 2011; WHO et al. 2011). At least one-third of HIV-infected persons worldwide are infected with Mycobacterium tuberculosis, and 8% to 10% of them develop clinical disease every year (Soumya et al. 2010). It results in 3 million deaths per year of which 0.5 million deaths occur among HIV-infected persons which accounted for almost 13% - 25% of global AIDS-related mortality (Linda-Gail and Robbin 2010; Stephen and Robbin 2011).

Incidence of tuberculosis: The annual incidence of TB in Ethiopia is estimated to be 168 cases per 100,000 population for smear positive tuberculosis, and 379 per 100,000 populations for all cases with over all prevalence of 643 per 100,000 populations. The TB/HIV co-infection rate is 40%-65% (Abera et al. 2009; Mekdes *et al* 2011) but from health facilities implementing TB/HIV services, only 24% HIV sero-prevalence is reported in TB patients (Aragie et al. 2012).

Risk factors: Studies done in Tanzania, Toronto and Uganda showed that health care delivery system, attitude of the community, level of HIV infection, CD_4 count, body mass index (marginally), marital status, sex, age, occupation and ART are the major factors that determine the incidence and mortality of HIV-associated TB (Macro et al. 2009, David et al. 2007; Richard et al. 2008*)*. Priorities in addressing TB-HIV co-infection include instituting the World Health Organization (WHO)'s '3Is' (Intensified TB case-finding, Isoniazid preventive therapy and Infection control) (Gysje et al. 2010), and Cotrimexazole prophylaxis (Global TB HIV working group of the stop TB partinership 2009*)*.

Several studies those have contradicted results were conducted in different countries on the incidence and associated factors of TB among patients in chronic HIV care units. In Ethiopia, although hospital-based cross sectional studies have reported that most of tuberculosis patients were co-infected with HIV, there is limited study conducted at East Gojjam zone though Amhara is the region most affected by HIV/AIDS (CSA 2012).

Materials and Methods

Study setting and population: A clinical based retrospective cohort study was carried out in East Gojjam zone, Northwest Ethiopia among pre- ART and ART clients enrolled between June 1, 2010 and May 30, 2012. Two hospitals and 14 health centers of the Zone were providing chronic HIV care services for12, 398 HIV infected people (East Gojjam zonal Health Department 2011).

Participants and data collection: All HIV clients those enrolled in the pre-ART and ART clinics of the Zone were considered as source population. All clients age greater than 15 who have been received chronic HIV care and HAART in pre ART and ART clinics within the stated duration were included in the study.

Sample size determination: Equation of two populations with equal sample size for difference of proportion was used to calculate the sample size. Patients on pre-ART were taken as non-exposed cohort and patients on ART were taken as exposed cohort. Epi Info version 3.5 2008 was used to calculate the required sample size for chart review. By taking confidence level 95%, power of the study 80%, expected frequency of disease in non-exposed group (cohort of pre-ART) 11.1% and relative risk (RR) 0.33 of (Arba Minch hospital), design effect 2, non-response rate10 %, total sample size was 964 with ratio of exposed to non-exposed 1:1

Six chronic HIV care units were selected by using simple random sampling technique since all care units are homogeneous in their all over provision of care. From the selected care units, charts of study subjects retrieved in the clinics were obtained proportionally by systematic random sampling procedure. For the randomly chosen chart that did not fulfill the selection criteria, the next chart was included in the sample. TB status among the pre-ART and ART clients were reviewed from records. The collected records were assessed based on the required measurements and variables of study. Ethical clearance was obtained from the Ethical Review Board of University of Gondar. Communication was made with concerned bodies. To keep confidentiality of any information provided regarding the study subjects, data collection procedure was anonymous.

Results

Socio demographic characteristics: A total of 964 client's charts (482 from each group) were included in the study. Among study subjects 197(40.9%) of pre-ART and 191(39.5%) of ART were found within the age range of 25-34 years. Four hundred thirty six (90.5%) of pre-ART and 410 (85.1%) of ART care attendants were followers of Orthodox Christian. Two hundred five (42.5%) and 227 (47.1%) of the pre-ART group and the ART group were married. Two hundred eighty three (58.7%) in pre-ART group and 266(55.2%) in ART group were not able to read and write (Table 1).

Clinical characteristics of study subjects: From the total of 964 chronic HIV care followers, 335(69.5%) of pre-ART and 251(52.1%) of ART had a functional status of "working". About 178(36.9%) of the pre-ART and 219 (45.4%) of the ART followers were WHO clinical stage III AIDS patients; 313(64.9%) of the pre-ART and 273(56.6%) of the ART care attendants had CD4 cells count <350cells/µl blood.

CPT had taken by 379 (78.6%) of the pre-ART and 358 (74.3%) of the ART care followers; and from those, 339(87.3%) pre-ART clients started Cotrimoxazole prophylaxis within six weeks of knowing their sero-status. INH prophylaxis therapy was used by 18(3.7%) of pre-ART and 10 (2.1%) of ART care followers. Body mass index was evaluated for 423(87.8%) of the pre- ART care attendants.

Table 1: Socio demographic characteristics of Pre ART and ART cohort in East Gojjam, Ethiopia, October 2012

Variable	Category	Number	
		Pre ART	ART
Age category	15-44	423(87.8)	296(61.4)
	45+	59(12.2)	186(38.6)
Sex	Male	161(33.4)	175(36.3)
	Female	321(66.6)	307(63.7)
Educational status	Not read and write	283(58.7)	266(55.2)
	Primary	103(21.4)	126(26.1)
	Secondary	61(12.7)	60(12.4)
	Tertiary	35(7.3)	30(6.2)
Religion	Orthodox	436(90.5)	410(85.1)
	Muslim	34(7.1)	58(12)
	Catholic	1(0.2)	12(2.5)
	Protestant	11(2.3)	2(0.4)
Marital status	Never married	117(24.3)	120(24.9)
	Married	205(42.5)	227(47.1)
	Widowed	108(22.4)	90(9.3)
	Divorced	52(10.8)	45(9.3)
Occupation	Civil servant	66(13.7)	82(17)
	Farmer	178(36.9)	171(35.5)
	House wife	66(13.7)	59(12.2)
	Commercial sex worker	41(8.5)	47(9.8)
	Merchant	71(14.7)	95(19.7)
	Student	36(7.5)	24(5)
	Others	24(5)	4(0.8)

Incidence of Tuberculosis: The overall cumulative incidence of tuberculosis was 6.2%, which was 8.1% in the pre-ART and 4.4% in the ART care followers. The highest incidence of TB was occur among females both in the pre-ART, 33(10.3%) and ART 15 (4.9%) groups. In both pre-ART and ART groups, clients with 15-44 years of age accounted the highest incidence of TB, which were 37(8.7%) and 18 (6.1%) respectively. It was utmost in individuals who were not able to read and write which was 32(11.3%) in the pre-ART and 13(4.9%) in the ART care followers. Concerning marital status, 19(17.6%) widowed from the Pre-ART group and 9 (10%) from ART group shared the peak burden of TB incidence.

Factors Associated with incidence of tuberculosis

The Pre-ART group: Incidence of tuberculosis had an association with clients' sex, educational status, marital status, functional status, baseline hematocrit, CD4 count, WHO clinical stage and Cotrimexazole preventive therapy (CPT). Males were less likely develop TB than females (AOR 0.312; 95% CI: 0.104, 0.939). Clients who were educated up to tertiary level had lower risk to develop TB than those who could not read and write (AOR 0.072; 95%CI: 0.007- 0.712). Widowed clients were 4 times more likely develop TB than those who never married (AOR 4.02; 95%CI: 1.222, 13.228). Bed ridden and ambulatory patients were 32.9 times (AOR 32.39; 95% CI: 8.079, 129.8) and 10.6 times (AOR 10.62; 95% CI: 3.756, 30) more likely to develop TB than those clients who were working respectively. Clients who had baseline hematocrit< 30% were 12.3 times more likely to develop TB than those had baseline hematocrit >30% (AOR 12.366; 95% CI: 4.75, 32.19). Clients with CD4 count <350 cells had 7.7 times greater risk for TB disease than those with >350 cells CD4 count (AOR 7.7; 95% CI: 2.378, 24.933). Those who were not on CPT had 5.89 times risk developing TB than those who were on therapy (AOR 5.896; 95% CI: 2.37, 14.664). Furthermore, those who were in WHO clinical stage IV had 16.29 times the risk of developing TB as compared to those who had WHO clinical stage I-III (AOR16.29; 95% CI:3.88, 68.412)(Table 2).

Table 2: Factors associated with TB in the Pre-ART group of chronic HIV care units, East Gojjam, North West Ethiopia, October 2012

Variable	Tuberculosis		95% CI	
	Yes (%)	No (%)	COR	AOR
Sex				
Male	6(1.24)	155(32.16)	0.34(0.12, 0.87)	0.312(0.104, 0.939)
Female	33(6.85)	288(59.75)	1	1
Hematocrit				
<30%	24(5)	119(24.7)	4.36(2.11, 9.06)	12.366(4.75, 32.19)
>30%	15(3.1)	324(67.2)	1	1
CPT				
Yes	21(4.36)	358(74.27)	1	1
No	18(3.7)	85(17.6)	3.61(1.75, 7.43)	5.896(2.37, 14.664)
CD4 count				
<350 cells/	33(6.85)	280(58.1)	3.2(1.25, 8.7)	7.7(2.378, 24.933)
>350cells/	6(1.24)	163(33.8)	1	1
Educational Status				
Not read and write	32(6.85)	251(52.1)	1	1
Primary	4(0.83)	99(20.5)	0.32(0.09, 0.97)	0.298(0.088, 1.01)
Secondary	2(0.42)	59(12.24)	0.27(0.04, 1.18)	0.248(0.049, 1.247)
Tertiary	1(0.21)	34(7.1)	0.23(0.01, 1.66)	0.072(0.007, 0.712)
Functional Status				
Bed ridden	9(1.87)	34(7.1)	5.28(1.98, 13.9)	32.39(8.079, 129.8)
Ambulatory	14(2.9)	90(18.7)	3.1(1.37, 7)	10.62(3.756, 30)
Working	16(3.3)	319(66.2)	1	1
Marital Status				
Never married	7(1.45)	110(22.8)	1	1
Married	11(2.3)	194(40.25)	0.89(0.31, 2.63)	1.05(0.313, 3.494)
Widowed	19(3.94)	89(18.5)	3.35(1.26, 9.25)	4.02(1.222, 13.228)
Divorced	2(0.42)	50(10.4)	0.63(0.09, 3.48)	0.704(0.117, 4.247)
WHO Clinical Stage				
Stage I,II, and III	31	433	1	
Stage IV	8	10	11.17(3.7, 33.57)	16.29(3.88, 68.412)

Statistical significance for AOR at less than 0.05

The HAART group

In the multivariate analysis clients' marital status, functional status, WHO clinical stage, CD4 cells count, CPT, and base line hematocrit had significant association with the incidence of TB (Table 3). Widowed individuals on ART were 6.68 times more likely on risk to develop TB than those who never married (AOR 6.688; 95%CI: 1.07, 41.66). Bed ridden patients on ART were 9.94 times more likely on risk to develop TB than those who were working (AOR 9.94; 95%CI: 2.287, 43.194). Individuals with baseline hematocrit of <30% had 5.928 times risk to acquire TB compared to those with baseline hematocrit of >30% (AOR 5.928; 95%CI: 1.488, 23.61). ART group with CD4 cells count less than 350 were found to be 10.056 times more likely on risk to develop TB than those with CD4 cells count > 350 (AOR 10.056; 95%CI: 1.262, 80.147). Individuals who were on WHO clinical stage IV were 3.878 times more likely to be co-infected compared to individuals who were on WHO clinical stage of (I-III) (AOR 3.878; 95%CI: 1.235, 12.177). CPT reduced incidence of TB by92.9% (AOR 0.071; 95% CI: 0.021, 0.242).

Table 3: Factors associated with TB in the ART group in chronic HIV care units of East Gojjam, North West Ethiopia, October 2012

Variable	Tuberculosis		95% CI	
	Yes (%)	No (%)	COR	AOR
Age				
15-44 years	18(3.7)	278(57.7)	3.95(1.08, 17.1)	2.589(0.592, 11.33)
45+ years	3(0.6)	183(38)	1	1
Marital Status				
Never married	2(0.41)	118(24.5)	1	1
Married	7(1.45)	220(45.6)	1.88(0.35, 13.29)	2.507(0.4, 15.707)
Widowed	9(1.87)	81(16.8)	6.56(1.27, 45.18)	6.688(1.07, 41.66)
Divorced	3(0.62)	42(8.7)	4.21(0.55, 37.57)	4.55(0.518, 40.046)
Functional Status				
Bed ridden	10(2.07)	76(15.8)	10.88(2.67,51.24)	9.94(2.287, 43.194)
Ambulatory	8(1.65)	137(28.4)	4.43(1.14, 23.35)	4.09(0.955, 17.585)
Working	3(0.62)	248(51.5)	1	1
Hematocrit				
<30%	18(3.73)	267(55.4)	4.36(1.19, 18.87)	5.928(1.488, 23.61)
>30%	3(0.62)	194(40.2)	1	1
CPT				
Yes	4(0.83)	354(73.4)	0.07(0.02, 0.23)	0.071(0.021, 0.242)
No	17(3.53)	107(22.1)	1	1
CD4 count				
<350 cells/	18(3.73)	253(52.5)	4.93(1.35, 21.35)	10.05(1.26, 80.14))
>350cells/	3(0.62)	208(43.1)	1	1
Duration of TB incidence on ART				
Within 6 months	15(3.11)	226(46.9)	2.59(0.55,16.77)	0.949(0.161, 5.565)
Within 6-12 months	4(0.83)	157(32.6)	0.99(0.15, 8)	0.338(0.08, 1.434)
After 1 year	2(0.41)	78(16.2)	1	1
WHO Clinical Stage				
Stage I, II and III	13(2.9)	424(88)	1	1
Stage IV	8(1.6)	37(7.5)	7.05(2.48, 19.72)	3.878(1.23, 12.177)

Statistical significant at p-value < 0.05

Discussion

The overall cumulative incidence of TB was 6.2%, which is in line with studies done in Kenya (6.2%) (NACC and NASCOP 2012), Uganda (6.4%)(William et al. 2011), but lower than the study conducted in other Sub-Saharan African countries which was >10% (Hayleyesus et al. 2010). Likewise, incidence of TB in the pre-ART group (8.1%) was lower than the study reported from Somaliland (>10%) (Ahmed 2008), and from another study conducted in Zimbabwe which was 25.3% (Elizabeth 2007).

Pre-ART: Incidence of tuberculosis among pre-ART clients was 8.1%, which is almost twice of those who initiated HAART (4.4%). The possible reason could be the fact that ART increases the immunity and decreases the viral load hence decreases the incidence of TB. But unmasking of the undiagnosed OI and ‘‘paradoxical’’ IRIS when an opportunistic infection (OI) diagnosed pre-ART initially responds to treatment but then deteriorates following ART initiation could be the possible reasons for TB after initiation of the ART(Lewis et al. 2012). The study showed that clients’ sex, educational status, marital status, functional status, Cotrimexazole prophylaxis, baseline hematocrit, CD4 cells count and WHO clinical stage had significant association with the incidence of tuberculosis in the pre- ART group. The risk to develop TB was higher in females than in males, which is similar to a survey done in Cape Town which showed women aged 15-24 have rates of TB 1.5–2-fold higher than men of the same age (Graeme et al. 2008; Theosmart 2012). But the present result is in contrast to study done in South West Ethiopia which was higher in males (Mohamed and Fasil 2011). Clients those not able to read and write had higher risk of developing TB than those who had primary educational level. Widowed individuals of pre-ART group were 4 times more likely to develop TB as compared to the unmarried. Bedridden and ambulatory patients had higher risk to develop TB than those who were working which is in line with a study done in Tanzania (Richard et al. 2008). Patients with CD4 cell count of <350cells/µl had more chance for the predictor of TB incidence which is consistent with a study done in South West Ethiopia (Mohammed and Fasil 2011) and other

studies carried out in eleven countries from different regions of the world (Amitabh and Suthar 2012; Tajelidin and Abdallah 2010; Stephen and Robbin 2011; Lewis et al. 2012) which a 5% decrease in TB disease risk for every increase in the CD4 count of 10 cells/mm^3, and an increase of 24% in the risk of TB disease for every year after HIV diagnosis (Tolu-Oni and Rolebohile 2011).

Pre-ART clients with low level of base line hematocrit level had more chance of developing TB than those with higher hematocrit level which is relatively similar to studies done in South West Ethiopia (Mohammed and Fasil 2011) and Durban (Lewis et al. 2012). CPT reduced incidence of tuberculosis among pre-ART cohorts by 94.1% which is similar to a study done in Kenya (92%) (American Medical Association 2011) but higher than a study conducted in Cote d'Ivoire (46%) (Elizabeth et al. 2008). Furthermore, those who were in WHO clinical stage IV had the risk of developing TB by 16.29% as compared to those who were WHO clinical stage (I-III).

ART: The cumulative incidence of TB among ART group was 4.4%, which is half of the pre-ART group in agreement with a study done in Kampala, Uganda (4.3%) (David et al. 2007, William et al. 2011), but lower than a study done in South Africa (24·7%) (Katharino et al. 2010). Incidence of TB in the ART group had significant association with marital status, functional status, Cotrimexazole prophylaxis, baseline hematocrit, CD4 cells count and WHO clinical stage. Widowed individuals of the ART group were 6.688 times more likely to develop TB as compared to the unmarried. Bedridden patients had 9.94 times higher risk to TB incidence than those who were working which is in line with a study done in Tanzania (Richard et al. 2008). CPT reduces TB incidence by 92.9% which was in line with a study done in different Sub Saharan African countries by the Johns Hopkins University (Timothy et al. 2010). Those who had base line hematocrit <30% had 5.928 times more likely of developing TB. Those who were WHO clinical stage IV AIDS patients were 3.878 times more likely to develop TB than those in WHO clinical stage (I-III) which coincides with the report of WHO (Mathew 2008). Patients with CD4 count <350cells/µl were more likely to develop TB which is similar to studies done in rural Northern Tanzania (Bernard et al. 2008), in nine Sub-Saharan African countries, and a report of International AIDS society conference (Soumya et al. 2010; Anthony et al. 2010; Benjamin et al. 2009).

Conclusion

In this study, incidence of TB was higher in the pre-ART cohorts than the ART cohorts that indicate HAART is strongly associated with a reduction in tuberculosis incidence. Marital status, functional status, base line CD4 cell count, base line hematocrit level, Cotrimoxazole prophylaxis therapy and WHO clinical stage had significant association with incidence of TB in both pre-ART and ART cohorts. However, sex and educational status had association only with those in the Pre-ART cohorts. Therefore, universal access to ART despite their WHO clinical stage, CD4 or viral load level is mandatory to reduce and to protect the co-infection of TB in individuals with HIV.

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Drug resistance pattern of diarrheogenic *Enterobacteriaceae* among HIV seropositive individuals on Cotrimoxazole prophylaxis at St. Paul Hospital, Addis Ababa

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Abstract
Back ground: Reduced efficacy of available drugs and anti-microbial resistance are challenges for control of infectious diseases. In recent years multi-drug resistant strains of bacteria has posed major public health problems. Hence prevention and control strategies should be implemented based on the studies available on pattern of drug resistance and prevalence of infectious agents particularly in resource limited countries, furthermore periodic surveillance of antibiotic resistance and effectiveness against common bacterial infections should be monitored.
Objectives: To assess antibiotic resistance pattern of diarrheognic *Enterobacteriaceae* among HIV infected ART-Naïve as well as ART-experienced individuals.
Methods: A hospital based cross-sectional study was conducted on individuals taking cotrimoxazole prophylaxis and presented with complaints of diarrhea. Stool samples were collected and examined by culture and sensitivity testing was done to evaluate antibiotic resistance patterns of selected diarrheogenic *Enterobacteriaceae*.
Result: From a total 162 HIV sero-positive cotrimoxazole taking patients presented with diarrhea, three types of bacterial isolates were observed in 14.8% of study participants. Most Prevalent bacterial isolates were *Shigella species* (6.8%), *Salmonella paratyphi* (4.9%) and *Salmonella typhi* (3.1%). Most frequently recovered bacterial isolates were resistant to co-trimoxazole with over all resistance pattern of (91.7%) and this isolates were also susceptible to Ceftraxone; next to Ceftraxone, the isolates was sensitive to Chloramphenicol (66.7%) than Naldixic acid and Nitroforantione. The most multi drug resistant bacteria isolates were *S.typhi* which is 100 resistant against Cotrimoxazole, Ampicilline, Nitroforantine and Naldixic acid. Despite high resistance pattern of Cotrimoxazole, statistically significant improvement of CD4 T cell level was seen after Cotrimoxazol prophylaxis and highly active anti-retroviral treatment, compared to baseline count with the mean difference of 169/mm^3 CD4 T-cell (paired t-test, $p<0.001$). WHO clinical stage, types of water source and raw vegetable consumption did not show a significant association with enteric bacterial infection and antibiotic resistance.
Conclusion: In present study, bacterial resistance to Cotrimoxazole was found to be very high; hence, its use for routine treatment of acute diarrheal illness is questionable.
Key words: Cotrimoxazole, diarrheognic *Enterobacteriaceae*

Introduction
According to recent estimate, 9 countries have more than 14% prevalence of HIV and 1.7 million HIV related deaths worldwide. Among this reported cases, most commonly infected were individuals at productive age thus results unprecedented burden on the economic growth of sub-Saharan countries (UNAIDS 2012). After introduction of ART and series of effort made to reduce the burden and incidence of the diseases, progressive decline of incidence and improvement of lives of cases with HIV/AIDS were reported in most countries: Contrary to the decline of HIV prevalence in some countries, new HIV infections continue to outpace those added on antiretroviral treatment. Particularly in sub-Saharan Africa where the prevalence is highest, increased death rate among midlife adults was evident in association with the fueling factor for HIV death such as co infection with TB and other Opportunistic infections (OIs) (Edith et al. 2008). The condition also aggravated by immune suppuration as a result of low CD4 count. Worldwide there are between 12 and 18 million HIV infected individuals with CD_4 cell counts of 200 cell/ micro liter or less those encountered one or more opportunistic related infectious diseases. The majority of these OIs were bacterial infections and intestinal parasites which cause life- threatening acute or chronic watery diarrhea (UNAIDS 2005). Pathogens such as *Salmonella* and non-typhoid *Salmonella*, which typically only causes enteritis in healthy individuals, can lead to severe septicemia in immune compromised patients (Jacobson et al. 1989). Episodes of bacterial enteritis in HIV infected patients with diarrhea, also contribute for mortality in HIV infected individuals (Nadelman 1985).

Reduced efficacy of available drugs and anti-microbial resistance is becoming challenges for control and prevention of infectious diseases in immune compromised hosts; in recent years the emergence and global dissemination of multi-drug resistant typhoidal strains of *Salmonella* has also posed major public health problems in developing countries. The condition is more aggravated by overuse, misuse, misdiagnosis and use of selected antibiotics for multiple infectious agents (Threlfall and Stoberingh 2000). The amplifying effect of antimicrobial therapy on resistance is also apparent with the transfer of resistance plasmids to previously susceptible strains (Kenneth J and Rayan C 2004). Due to its proven effect on OIs, Cotrimoxazole (800mg SMZ + 160mg TMP) prophylaxis was been recommended for high risk group of patients (WHO/UNAIDS 2000) (Carlucci et al. 2008). It is provided routinely after emergence of AIDS pandemic in most developed world following the promising findings of two studies in West Africa (Anglaret et al. 1999). On the other hand wide spread use of Cotrimoxazole antimicrobial agent as prophylactic drug has shown negative impact on the control of microbial resistance. Extended use would promote corresponding population-level resistance with resultant loss of the utility of TMP-SMZ to treat life-threatening bacterial infections (Mermin et al. 2004). Daily TMP-SMZ may reduce its susceptibility to commensal intestinal *Escherichia coli,* increasing the burden of TMP-SMZ-resistant pathogens. Data shows prevalence of TMP- SMZ resistance ranged from (71%) to (81%) after 2 weeks use of this drug as a prophylaxis. It is also evident from similar findings, TMP-SMZ-resistant *E. coli* increased significantly from 78% to 98% among persons taking daily TMP-SMZ (Chiller et al. 2009).

Bacterial diseases and drug resistance control strategies should be implemented based on the studies on the pattern of drug resistance in a given categories of patients with certain disease condition; furthermore, periodic surveillance of antibiotic resistance and effectiveness of available antibiotics against common bacterial infections should be monitored. In Ethiopia, studies in the area of microbial drug resistance are much more limited, even most studies didn't consider the prevalence after intervention with some kind of chemoprophylaxis like Cotrimoxazole (TMP-SMZ) and isoniazid (INH) in association with different risk factors. Therefore the present study was conducted to see drug resistance pattern of selected diarrheogenic *Enterobacteriaceae* among Cotrimoxazole prophylaxis's taking HIV infected ART-Naïve as well as ART-experienced individuals on ART taking Cotrimoxazole prophylaxis at St. Paul's Hospital Millennium Medical College ART Clinic.

Materials and Methods

***Study design*:** A cross-sectional hospital-based study was done at St. Paul's Hospital Millennium Medical College from December 1, 2013 to March 31, 2014 after approved by the study area IRB. Written consent was obtained from each participant and laboratory results were communicated with the physicians in charge.

Sample collection, inoculation and identification: Stool samples were collected and inoculated into labeled enrichment culture medias. The standard bacteriological culture methodology was followed for stool culture, which involved inoculating a sample into selective enrichment broth medium and sub culturing in to differential and selective agar media followed by incubated at 37°C for 18 to 24 hours. The sample inoculums were read based on the colony characteristic chart. Biochemical tests were done to confirm *Salmonella spp*. as per bacteriology technical manual (Monica C 2000).

***Antimicrobial susceptibility test*:** Susceptibility tests were performed using standard Modified Kirby-Bauer Disc Diffusion susceptibility testing technique against the following antimicrobial agents: Chloramphenicol, Trimethoprim/Sulfamethoxazole, Ciprofloxacin, Ceftriaxone, Naldixic acid, Nitroforantion, and Ampicillin. The test was performed on Mueller-Hinton agar medium. The turbidity of nutrient broth was standardized equivalent to 0.5 McFarland standards (Monica C 2000). The diameter of the zone of inhibition around the disc was measured to the nearest millimeter, inhibition zone diameters for each organism fall within the limits of the standard. The interpretation of the findings was done by referring to the table of critical diameters classified as sensitive and resistant according to National Committee for Clinical Laboratory Standard (NCCLS 2005). Quality of Bacteriological analysis checked by Control strains to check functionality of different media, incubating conditions and biochemical media with bacterial strain of *E. faecalis* ATCC 29212/33186.

***Data analysis*:** The data was analyzed using SPSS version 18.0 software and level of resistance was summarized in percentages for each isolated bacteria. Multivariate analysis was calculated for associated factor. Odds of bacterial isolates and chi-square or student T test were used. P-value of < 0 P-value of < 0.05 was considered significant within 95% CI.

Results

Demographic Characteristic: From the total 162 patients participated in the study, 158 (97.5%) of them were from Addis Ababa. Ninety seven percent of the study participants fall between the age group of 26-45 years old and the median age were 36 years old (Table 1). From total study participants, 120 (74.1%) were on Cotrimoxazole (800mgSMZ+ 160mg TMP) prophylaxis and highly active anti-retroviral treatment (HAART) and females comprised 78 (48.1%). The remaining 42(25.9%) were on pre ART chronic follow up care with 32(19.7%) female. Seventy six percent of study participants were under Cotrimoxazole prophylaxis for six and more. Fifteen percent of study participants were smear positive TB patients. Around 10.4%, 7.4% and 3% of study participants were on INH, both on INH and HAART, and INH and Cotrimoxazole prophylaxes respectively.

BaselineCD$_4$, current CD$_4$ and risk factor: About sixty percent of study participants base line CD4 count were below 200/mm^3 with a mean CD4 count of 175 cell/mm^3 (Table 2). After HAART and Cotrimoxazole prophylaxis, significant improvement of CD4-T cell level was seen compared to baseline count with the mean difference of 169/mm^3 CD4 T-cell (paired t-test, p<0.001).

Table 1: Age and Sex distribution of study participants, St .Paul's Millennium Medical College, in 2014

Characteristic	CD4 T-cell value(mm^3)	frequency	Percentage
Baseline CD4	<100	45	27.8
	101-200	52	32.1
	201-250	23	14.2
	251-350	40	24.7
	>350	2	1.2
Current CD4	<100	14	8.6
	101-200	24	14.8
	201-250	15	9.3
	251-350	37	22.8
	>350	72	44.4
Total		162	100.0

Table 2: Baseline and current CD4 T-cell counts, St. Paul's Millennium Medical College, 2014

Characteristic		Total	Frequency (%)
Sex	Male	52	(32.0)
	Female	110	(67.9)
Age	18-25	15	(9.3)
	26-35	58	(35.8)
	36-45	58	(35.8)
	46-55	20	(12.3)
	56-65	8	(4.9)
	>66	3	(1.9)
Total		162	100

Majority (97.5%) of study participants had access for toilets and piped water supply. Nearly half (48.8%) and 44.4% of them had regular habits of drinking treated water with chlorinating agent and raw vegetables consumption respectively.

Bacterial isolates and resistance pattern: Of 162 stool culture and drug sensitivity testing, 24(14.8%) diarrhea-causing *Enterobacteriaceae* were isolated excluding *E.coli*. The prevalent isolates were *Shigella spp*, *Salmonella paratyphi* and *Salmonella typhi* respectively (Table 3). Antimicrobial drug sensitivity of isolates showed high level of resistance to the majority of the drugs tested. Most bacterial isolates were resistance against Cotrimoxazole with 87.5% minimum resistance pattern observed for *S.para typhi* and 100% resistance pattern for S. *typhi*. The overall resistance patterns of the isolates against this antimicrobial agent were 91.7%. Next to Cotrimoxazole, *S. paratyphi* and *S. typhi* isolates were 100% resistance against Ampicillin (Table 4).

Table 3: Prevalence of diarrheogenic *Enterobacteriaceae,* St. Paul's Millennium Medical College, in 2014

Bacterial isolates	Frequency	Percent
No growth	139	85.8
S. paratyphi.	8	4.9
S. typhi.	5	3.1
Shigella spps.	10	6.2
Total	162	100.0

Ceftriaxone was sensitive for Shigella spp. and *S. para typhi* with the sensitivity of 90.9% and 62.5% respectively and showed overall resistance pattern of only 20.8%. Next to ceftriaxone, Ciprofloxacin and Chloramphenicol were sensitive for *Shigella* spp with the same cumulative resistance patterns of 33.3%. *S.typhi* was found to be the most multi drug resistance isolates with 100% resistant to Cotrimoxazole, Ampicillin, Nitroforantine and Naldixic acid. In addition, it showed 80% resistant to Chloramphenicol. Isolates of *S.para typhi* also shows 100% resistance to Ampicillin and Naldixic acid. *Shigella species* isolates were susceptible for most antimicrobial agent with 100% to Chloramphenicol (Table 4). Regarding the risk factors assessed, toilet use habits, type of water source, raw vegetable consumption and WHO clinical stage has no significant association with wide resistance pattern of the isolates.

Table 4: Antibiogram patterns of some diarrheogenic *Enterobacteriaceae* isolates, St. Paul's Hospital Millennium Medical College, in 2014.

Antibiotic& potency used/ µg concentration	Sensitivity Pattern	Organisms isolated						
		S.paratyphi		*shigella spps.*		*S.typhi*		Total
		No	%	No	%	No	%	R%*
Trimethoprim-1.25	Resistance	7	87.5	10	90.9	5	100.	91.7
Sulfamethoxazole23.75	Sensitive	1	12.5	1	9.1	0	0.0	
Ampicillin(10 µg)	Resistance	8	100.0	9	81.8	5	100.0	91.7
	Sensitive	0	0.0	2	18.2	0	0.0	
Chloramphenicol (30 µg	Resistance	4	50.0	0	0.0	4	80.0	33.3
	Sensitive	4	50.0	11	100.0	1	20.0	
Nitroforantion(30 µg	Resistance	5	62.5	3	27.3	5	100.0	54.2
	Sensitive	3	37.5	8	72.7	0	0.0	
Naldxic Acid(30 µg)	Resistance	8	100.0	4	36.4	5	100.0	70.8
	Sensitive	0	0.0	7	63.6	0	0.0	
Ceftriaxone(30 µg)	Resistance	3	37.5	1	9.1	1	20.0	20.8
	Sensitive	5	62.5	10	90.9	4	80.0	
Ciprofloxacin(5 µg)	Resistance	5	62.5	2	18.	1	20.	33.3
	Sensitive	3	37.5	9	81.8	4	80.0	
Total Isolates		8		11		5	N=24	

R% total frequency of resistance*

Discussion

In this study, drug resistance pattern of some diarrhea-causing *Enterobacteriaceae* other than *E.coli* in relation to CD4 T lymphocytes counts, WHO clinical stage and Cotrimoxazole prophylaxis were assessed. Resistance pattern of some *Enterobacteriaceae* isolates against Cotrimoxazole was 87.5%, 90.9%, and 100% for *S.para typhi*, *Shigella* spps and *S.typhi* respectively which is in line with a study conducted in Zambia (90%) (Mwansa et al. 2002) but lower than a report from Uganda for both *Shigella* spp. This study showed over all resistance patterns of 91.7% which was higher than similar studies conducted in Northern Ethiopia (76.7%) (Asseffa et al. 1997, Huruy et al. 2011) and a study done in Uganda (83%). This periodic increment of resistance isolates with low CD4 count was also reported from Kenya (98%) (Mary et al. 2008); this might be associated with selection pressure of more resistance isolates or transfer of resistance gen between microbes (Kenneth J and Rayan C 2004)). This periodic increment of resistance isolates with low CD4 count was also reported from a review of resistance to Nalidixic acid and Ciprofloxacin in Asia countries (64.5%) (Bing et al. 2012). Ampicillin were also found to be 100% resistance to *S. para typhi* and

S.typhi but 80.8% for isolates of Shigella spp which was similar to findings from Northern Ethiopia (Huruy et al. 2011). Unlike wise, Ceftraxone was sensitive for *Shigella* (90.9%) and S.*paratyphi* (62.5%) with overall sensitivity pattern of 79.2% which is lower than a study conducted in India (94%) for *S.paratyphi*. Ciprofloxacin and Chloramphenicol were found to be 81.8% and 100% sensitive for *Shigella* isolates which was lower than a finding in Rourkala India 98% and 76.6% respectively (Bhattacharya 2011) but higher than another study in Ethiopia (51.7%) for Chloramphenicol (Huruy et al. 2011). *S.typhi* were found to be the most multi drug resistance isolates with 100% resistant to Cotrimoxazole, Ampicillin, Nitroforantine and Naldixic acid which was higher than a similar findings done in Ethiopia which was only 33.3% (Huruy et al. 2011) and India (12.7%). Resistance pattern for both *S. typhi* and *S. paratyphi* against Ciprofloxacin was also reported to be less than 2 %. *Shigella spp* isolates were found to be susceptible for most antimicrobial agent; 100% to Chloramphenicol and 9.1% to Cotrimoxazol which was similar to different reports else were (Threlfall and Stoberingh 2000). The overall high resistance pattern observed here could be due to overuse of this selected drug within the community. Despite extensive bacterial resistance was observed against most antibiotics, good restoration of immunity with significant improvement of CD_4 T cell level were observed in individuals who were under Cotrimoxazol prophylaxis and HAART for at least six months. It was in line with the report of most studies (Vajpayee et al. 1999, WHO 2006, WHO 2005).

Conclusion

In this study, extensive resistance pattern of diarrheaognic *Enterobacteriaceae* were observed against the antimicrobial agents of Cotrimoxazole, Ampcilline, Naldixic acid and Nitoforantion. Routine treatment of acute diarrheal illness with Cotrimoxazole was questionable due to the development of high resistance pattern. Ceftriaxone and Chloramphenicol were found to be the most and relatively good drugs against common microbial isolates respectively.

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Assessment of national tuberculosis and HIV collaborative program implementation status in health care settings of Ethiopia

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Abstract

Background: Ethiopia has adopted the World Health Organization recommendation for TB and HIV collaborative activities since 2004. These collaborative activities have been scaled up in a phased manner and covered large number of health facilities across the nation. However, there is scarcity of information on implementation of these collaborative activities in Ethiopia.

Objective: To assess the status of implementation of TB and HIV collaborative activities in health facility settings of Ethiopia.

Methods: A cross sectional study mainly quantitative supplemented by qualitative methods was undertaken from May 10 to July 10, 2014 in 132 selected health facilities. Statistical analysis was performed using SPSS version 20.

Result: About 81% of the respondents in the selected health facilities reported the screening of People Living with HIV in care for TB at every follow up visit, whereas, only 28.7% of those health facilities reported the screening of PLWHIV for TB at enrolment to HIV chronic care. About half of the public health facilities assessed were not implementing Isoniazid Preventive Therapy and only 18.2% of eligible clients were getting this Preventive Therapy. Among the co-infected patients, 32% were not linked to chronic care services and 45.3% were not getting ART during TB treatment. On the other hand, about two thirds of the co-infected patients are getting the Cotrimoxazole Prophylaxis Therapy.

Conclusion: Most of anti-TB and HIV collaborative activities were not implemented as expected in the health facilities. Thus it needs integration from the ministry to the health facilities level in order to improve the collaborative activities.

Key words: Tuberculosis screening, Ionized Prophylaxis Therapy, Cotrimexazole Prophylaxis Therapy, people living with HIV

Introduction

Tuberculosis (TB) and Human Immuno deficiency Virus (HIV) are major sources of morbidity and mortality throughout the world (WHO 2015). An estimated 10.4 million new TB cases and 1.8 million deaths, including 0.4 million HIV co-infected people occurred in 2015 (WHO 2016b) and there were an estimated 2.1 million new HIV infections worldwide, adding up to a total of 36.7 million [34.0 million–39.8 million] people living with HIV occurred in 2015 (WHO 2016a). In addition, 1/3 of the worldwide population is latently infected with *Mycobacterium tuberculosis* (Mtb). Mtb is an intracellular pathogen capable of infecting and surviving within the host's mononuclear cells, particularly macrophages (Crevel et al 2002). HIV is the strongest risk factor for developing TB in those with latent or new Mtb; increasing the risk of developing TB by 20 to 37 fold in people living with HIV (PLWHIV) compared to those who do not have HIV infection (WHO 2011). In view of the worsening of HIV/AIDS and TB situation in the world and taking into account the close association between the two diseases, WHO has issued a policy recommendation to address the dual epidemic in 2004. The national TB prevention and control program recommended TB screening to be integrated in every services outlet of the health facilities. It is also indicated that all HIV positive clients should be screened for TB symptoms both at the time of enrolment as well as at every follow up visit (FMOH 2014). Since then Ethiopia adopted the TB/HIV collaborative initiative and started to pilot it in nine health facilities. Subsequently, the national program developed a national TB/HIV guideline to standardize the implementation of TB/HIV collaborative activities and expand it nation-wide and all packages of the TB/HIV collaborative initiatives have been implemented in the country.

The implementation status of the national TB prevention and control program in Ethiopia had been reviewed and this recommended assessing the adequate coverage in the National TB strategic plan and wide implementation of the TB/HIV collaborative activities at the service delivery points. There is scarcity of data on the seven core indicators of the TB/HIV collaborative activities and only two of these have been captured and reported by the Health Management Information System (HMIS) until recently. Besides, the quality of TB/HIV information generated by HMIS is a matter of concern. Therefore, this study aimed to assess the status of implementation of TB/HIV collaborative activities and to evaluate the extent of implementation on determinants of Intensified Case Finding (ICF) which is IPT, CPT and ART coverage among People Living With HIV (PLHIV) in Ethiopia.

Materials and Methods

***Study design, study setting and study population**:* A cross sectional study design and multi stage cluster sampling method was used. A total of 132 public health facilities (33 Hospitals and 99 Health centers) from 126 hospitals and 2371 health centers those providing TB Directly Observed Therapy, Short Course (TB DOTS) service were included. Randomly selected records of HIV positive TB patients on chronic care from July 1, 2013 to March 31, 2014, TB and ART focal persons in the health facilities were the study populations.

Sample size Determination: The sample size for patient record review was determined assuming a prevalence of 32% IPT uptake among HIV infected clients, free of active TB, from previous study. With a 95% certainty and a maximum discrepancy of 6% between the sample and the underlying population were considered for all objectives. An additional 10% non-response rate was considered as a contingence and a design effect of 2 was taken for the multistage nature of the sampling. And accordingly, the required minimum sample size for IPT was 511 per reporting domain. The sample size for pastoralist and urban was 511 each, while for the Agrarian was 1,178 with the overall sample size of 2,200 patient records. At the health facility, all patients' record were listed and numbered consecutively during the enrolment time. The number of patients was determined by starting randomly and review the records systematically based on the total number listed. Two key informant interviews (KII) were administered for TB and ART focal persons in the selected facilities. Pretested standard and structured questionnaires for the quantitative data and semi-structured questionnaires for the qualitative (KII) data were used. Data were double entered and analyzed using Census and Survey Processing System (CSPro) and SPSS version 20 respectively. The survey was conducted after getting an approval from the Institutional Review Board of EPHI.

Results

Implementation of ICF: Among the 2,161 HIV infected clients enrolled in the study, 1880 (87%) were screened for TB during their last follow up visit. There was no major variation in screening coverage across the different agro-ecological settings. However, public health centers perform better in terms of TB screening coverage compared to hospitals (87% vs. 83%). About 81% of the respondents in the selected health facilities reported screening of PLHIV in care for TB at every follow up visit; On the other hand, only 28.7% of health facilities reported screening of PLHIV for TB at enrolment to HIV chronic care. Six percent of the respondents in the selected health facilities reported screening of PLHIV only when patient complains of TB symptoms. Fifty three percent of the health facilities were not adhering to the recommended guidance for TB screening among PLHIV. Forty one percent of the health facilities/ART clinics had the current national guideline (Programmatic management of TB, TB/HIV and Multi Drug Resistant TB, 2012) at the time of visit and from those only 19.5% of the facilities adhered to the nationally recommended guideline for investigating TB screening positive PLHIV to confirm/rule out TB.

Isoniazid Preventive Therapy (IPT): Out of 87 ART health facilities, only 50% of them (11 hospitals and 33 public Health Centers) were providing IPT service. Among 1880 PLHIV screened for TB, 184 (9.8%) were found to have presumptive TB); while the incidence of TB among HIV positive clients enrolled to the study was 4.5% (Figure 1).

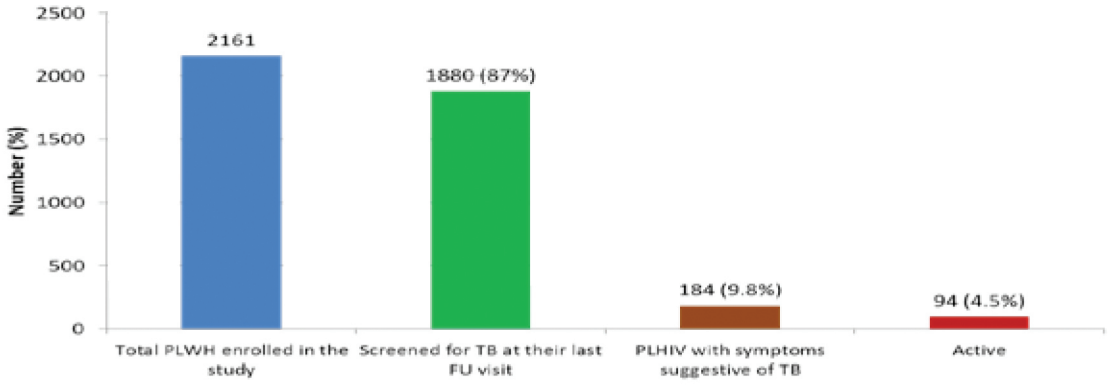

Figure 1: proportion of PLHIV under chronic care screened for TB symptoms and diagnosed with active TB

Among the 1,696 PLHIV screened and found to be negative for TB, only 308 (18.2%) had started IPT. The implementation of IPT is low in Pastoralist compared to urban setting and there was no significant difference in IPT implementation between Urban and Agrarian settings. Among the PLHIV clients enrolled to IPT, 105 were eligible for evaluation and the IPT completion rate was 64.8% (Table 1). Only two clients who started IPT have developed active TB disease during the course of their treatment.

Table 1: IPT completion rate in different agro-ecologic setting and health facility type

Variable		N (%)	IPT completion	
			OR (95%CI)	P-value
Agro ecological setting	Urban	14 (20.9)	1	
	Agrarian	41 (61.2)	0.72 (0.48,1.07)	0.106
	Pastoralist	12 (17.9)	0.56 (0.32,0.98)	0.046
Health Facility type	Public hospital	8 (11.8)	0.36 (0.128,1.013)	
	Public health center	60 (88.2)	1	

The major barrier for the IPT implementation as reported by respondents from selected health facilities was INH supply interruption 84.2%.

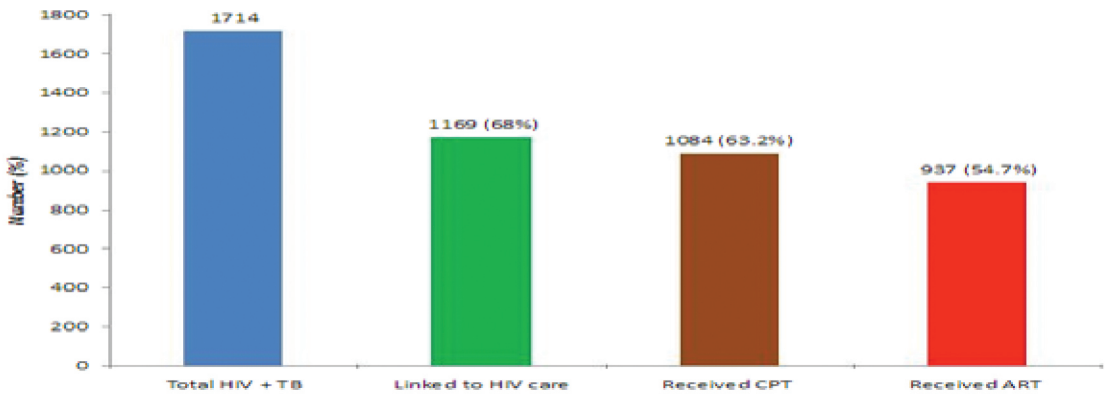

Figure 2: TB patients co-infected with HIV linked to chronic HIV care service and received ART and CPT

Patient's residence, sex and having history of previous treatment were found to be the independent predictors of ART initiation among TB patients co-infected with HIV (Table 2).

Table 2: Socio-demographic and clinical factors associated with ART uptake in TB/HIV co-infected patients

Parameters		N (%)	AOR (95% C.I.)	P-value
Residence	Urban	1290 (86.3)		
	Rural	204 (13.7)	0.62 (0.50 ,0.76)	<0.001
Sex	Male	740 (49.5)		
	Female	754 (50.5)	1.34 (1.09 ,1.65)	0.006
Age	0-14	74 (5)		
	15-44	1194 (79.9)	1.00 (0.50 , 2.06)	0.980
	45+	226 (15.1)	1.86 (0.87, 4.00)	0.110
Weight	<29 kg	72 (4.8)		
	30-39 kg	196 (13.1)	0.74 (0.37, 1.51)	0.411
	40-54 kg	879 (58.8)	0.78 (0.38, 1.57)	0.480
	55+ kg	347 (23.2)	0.60 (0.29, 1.25)	0.171
Type of TB	Pulmonary Positive	327 (21.9)		
	Pulmonary Negative	719 (48.1)	1.12 (0.86, 1.40)	0.428
	Extra pulmonary	448 (30)	1.12 (0.84, 1.49)	0.443
Treatment History	New	1279 (85.6)		
	Retreatment (R,F,D)	66 (4.4)	2.46 (1.41, 4.31)	0.002
	Others(O)	92 (6.2)	1.83 (1.09, 3.08)	0.022
	Transfer in (T)	57 (3.8)	1.52 (0.99, 2.31)	0.055

Patient's residence, sex, and being in the age group of 15-44 years were found to be the independent predictors of CPT uptake among TB patients co-infected with HIV (Table 3).

Table 3: Factors associated with CPT initiation for TB patients co-infected with HIV

Parameters		N (%)	AOR (95% C.I.)	P-value
Residence	Urban	1291(86.4)	0.621 (0.5 , 0.77)	<0.001
	Rural	204 (13.6)		
Sex	Male	741(49.6)		0.019
	Female	754(50.4)	0.775 (0.626 , 0.96)	
Age	0-14	74(4.9)		
	15-44	1195(79.9)	0.295 (0.119 , 0.731)	0.008
	45+	226(15.1)	0.453 (0.175 , 1.173)	0.103
Weight	<29 kg	72(4.8)		
	30-39 kg	197(13.2)	0.746 (0.327 , 1.706)	0.488
	40-54 kg	879(58.8)	0.626 (0.278 , 1.412)	0.260
	55+ kg	347(23.2)	0.557 (0.241 , 1.287)	0.171
Type of TB	Pulmonary Positive	327(21.9)		
	Pulmonary Negative	720(48.2)	1.267 (0.977 , 1.644)	0.074
	Extra pulmonary	448(30.0)	0.921 (0.69 , 1.229)	0.577
Treatment History	New (N)	1280(85.6)		
	Retreatment (R,F,D)	66(4.4)	1.264 (0.736 , 2.171)	0.397
	Others(O)	92(6.2)	1.246 (0.739 , 2.101)	0.409
	Transfer in (T)	57(3.8)	1.223 (0.789 , 1.896)	0.369

Discussion

This study assessed the status of implementation of TB/HIV collaborative activities including IPT, CPT and ART coverage among PLHIV in Ethiopia. The finding revealed that only 19.5% of health facilities were strictly adhered to the national guidelines for TB-HIV collaborative activities. This might be due to lack of motivation and knowledge gap of the health workers. TB screening for HIV patients was much lower (28.7%) compared to follow up visits. This could be related to the unavailability and lack of motivation for use of revised national guideline for programmatic management of TB, TB/HIV and Multi Drug Resistant TB (MDR TB). More than 59% of the public health facilities were not used the revised National Guideline at HIV care units and this is in line with external midterm review of the TB program report (unpublished result). The IPT uptake among the eligible PLHIV in this study was higher (18.2%) compared to national TB/HIV sentinel surveillance report of 2013 (Denegetu and Dolamo 2014); while lower compared to the finding conducted in six health facilities (Wesen and Mitike 2012) and study conducted in a teaching hospitals of Addis Ababa (Kassa et al. 2012a) which could be attributed to Isoniazid supply interruption and stock out.

This is in contrast to previous literature, which focuses on the confidence of health workers in prescribing INH prophylaxis for eligible PLHIV (Kassa et al. 2012a). The implementation of IPT was lower (17.9%) in pastoralist areas, which could be due to poor access to health services relatively by pastoralist community. This study showed that a relatively high proportion (64.8%) of patients who commenced treatment for LTBI completed the six month scheduled course compared to other studies (Goswami et al. 2012; Diaz et al. 2010). However, the completion rate is lower compared to the studies conducted in Australia (Dobler and Marks 2012) and Italy (Codecasa et al. 2013).

One third of co-infected patients were not linked to the chronic HIV care and treatment service. This might be related to supply interruption, knowledge gap and lack of staff motivation. ART uptake coverage for TB-HIV co-infected patients was lower (54.7%) than the global recommended (FMOH 2014; Schutz et al. 2010; WHO 2010) and other African countries (Pepper et al. 2011; Tayler-Smith et al. 2011) and India (Njozing et al. 2010) but similar to the 2013 national surveillance report in Ethiopia (EPHI 2013) and another study in Cameroon (Njozing et al. 2010). Patients from rural, female and previous treated patients had low proportion of uptake of ART relative to patients from urban settings, males and new TB patients respectively. This is similar with previous report in Cameroon (Njozing et al. 2010), in South Africa (Pepper et al. 2011). The coverage of CPT for TB patients co-infected with HIV in this study were lower (63.2%) compared from global recommended and target (FMOH 2014). However, this finding is higher than the figure reported in a study in Cameroon (Njozing et al. 2010) and Addis Ababa (Denegetu and Dolamo 2014); while much lower than the CPT Coverage reported in a study from India (Dave et al. 2014). In this study, initiation of CPT was affected by Patient's residence, sex, and age; females, which is similar with previous study in Cameroon (Njozing et al. 2010). Poor documentation of data regarding linkage for care, CPT and ART coverage could be another factor for the low ART coverage.

Conclusion

The practice of TB screening both at enrolment and every follow up visit of HIV care, and the care and treatment coverage of IPT, CPT and ART uptake for TB-HIV patients were very low at both public hospitals and health centers of Ethiopia. Therefore, higher officials should give stress on the major barriers of TB-HIV collaborative activities i.e. accessibility of health service, drug supply interruption and lack of training of health worker.

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Conventional and molecular methods for the diagnosis of smear negative pulmonary TB and concordance of empirical TB diagnosis with culture assay

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Abstract
Background: Ethiopia is among high TB burden countries. The proportion of smear negative patients among pulmonary TB cases was 51%. Nevertheless, microscopy is still a primary tool for TB diagnosis. In the absence of sensitive diagnostic methods, clinicians often make the diagnosis of smear-negative pulmonary TB with information obtained through the clinical history, physical examination and chest X-ray. The treatment of smear negative TB patients with those criteria largely depends on the treating clinician. There is limited data on the utilization of clinical criteria commonly used to initiate TB treatment empirically when culture is used as the reference method. Beside this, there are a number of sensitive diagnostic methods that have a substantial contribution for the diagnosis of smear negative TB patients, but only few studies were conducted in Ethiopia to address this issue.
Objective: To assess the contribution of conventional and molecular methods for smear-negative patients and describe the concordance rate of empiric TB treatment with culture assay.
Methods: A cross-sectional study was designed on smear negative TB presumptive patients referred to St. Peter's TB Specialized Hospital from December 2014 to June 2015. Consecutive smear negative TB presumptive patients, aged greater or equal to 15 and willing to participate were included in the study. Socio-demographic and clinical data were collected using the data collection form designed for the study purpose. The data collection form was designed to capture patient demographic data, signs and symptoms, chest X-ray findings and empirical TB treatment initiations. Spot-Moring-spot sputum was collected using sterile falcon tubes for routine diagnostic purpose. Direct ZN examination was done on Spot-Moring-spot sputum at St. Peter's TB Specialized Hospital**.** The morning sputum was used for culture (LJ and MGIT), TB-LAMP, Xpert MTB/RIF assay and fluorescent microscopy examination. Data were captured and analyzed using SPSS.
Results: This study enrolled 459 smear negative presumptive TB patients. Most (57%) of the study participants were female; with median age of 40 IQR (28–55) years. HIV test results were available for 41% of the study participants and the prevalence of HIV among the study participants was 30%. Three hundred eighty three cases were having both treatment and lab results. Forty six cases were treated empirically. The sensitivity and specificity of empiric TB treatment when compared to culture were 45.8% and 90% respectively. The overall culture positivity rate was 6.8% (30/439), of which 6.6% (26/391) was by MGIT and 5.3% (23/436) was by LJ method. Direct and concentrated fluorescent microscopy adds 0.9% and 1.3% detection rate compared to the direct ZN. The overall sensitivity and specificity of TB-LAMP was 61.5% (16/26) and 96.6% respectively. The overall sensitivity and specificity of Xpert MTB/RIF was 70.8% (17/24) and 97.2% respectively.
Conclusion: TB-LAMP and Xpert MTB/RIF assay can provide confirmatory results for at least two third of TB cases.
Key words: TB-LAMP, Xpert, smear negative TB

Introduction
Globally, 9.6 million people are estimated to have fallen sick with tuberculosis (TB). In 2014, 6 million new TB cases were reported to the World Health Organization (WHO) and 5.2 million of them were pulmonary TB. Among the 5.2 million pulmonary TB patients, 42% of patients were diagnosed based on the information obtained from clinical evidences (WHO 2015). In Ethiopia, TB is a major public health problem and WHO categorizes Ethiopia as a high TB and multi-drug resistant TB burden country. According to the WHO annual report, the proportion of smear negative patients among pulmonary TB cases was 51% (WHO 2015). Even if majority of the patients are smear negative TB cases, microscopy is still the primary tool for TB diagnosis; the method has low sensitivity and can only detect 36 % of tuberculosis cases (Dagnaw et al. 2014). Clinicians often make the diagnosis of smear-negative pulmonary TB with information obtained through the clinical history, physical examination and chest X-ray (Siddiqi et al. 2003, Soto et al. 2013). The treatment of smear negative TB patients with the criteria according to the national guideline largely depend on the treating clinician. Even if half of the pulmonary TB patients are diagnosed on clinical evidences, there

is limited data on the utilization of clinical criteria commonly used by clinicians to initiate empirical TB treatment when cultures are used as the reference method. Currently, there are a number of sensitive diagnostic methods that have a substantial contribution for the diagnosis of smear negative TB patients. Fluorescence microscopy improves the diagnostic yield, around 10% increase in sensitivity than conventional ZN microscopy. Xpert MTB/RIF assay has 67% pooled sensitivity for smear negative TB patients (Steingart et al. 2014). TB-loop mediated isothermal amplification (LAMP) assay adds on test for smear negative TB patient has 48.8% to 55.6% sensitivity (Boehme et al. 2007, Mitarai et al. 2011, Ou et al. 2014).

Few studies were conducted in Ethiopia on the contribution of fluorescence microscopy and Xpert MTB/RIF assay for the diagnosis of smear negative TB patients. Two studies conducted in Addis Ababa indicated the detection rate of fluorescence microscopy 17.7% from all referred TB presumptive cases and 8.4% from HIV positive individuals (Getachew et al. 2015 and Solomon, et al. 2015). The values of Xpert MTB/RIF assay were evaluated at Karamara Hospital on consecutively recruited TB presumptive patients and at five Health Centers around Adama on HIV-positive adults. The sensitivity of Xpert MTB/RIF assay for smear negative and culture positive was 48.6% for TB presumptive patients and 57.4% for HIV-positive adults (Geleta et al. 2015; Balcha et al. 2014). There is no data on the diagnostic yield of TB-LAMP. Therefore, the present study was designed to assess the contribution of TB-LAMP as well as Xpert MTB/RIF and fluorescence microscopy for diagnosis of smear negative TB and describe the concordance rate of empiric TB treatment with culture.

Materials and methods

A cross-sectional study was designed to evaluate the contribution of conventional and molecular methods for the diagnosis of smear negative pulmonary TB patients and assess the concordance rate of empirical TB treatment with culture results. The study was conducted on smear negative TB presumptive patients referred to St. Peter's TB Specialized Hospital (SPH) from December 2014 to June 2015. Smear negative TB presumptive patients, age greater or equal to 15, referred to SPH during the study period were included in the study. All TB presumptive patients referred during the intake period and those who were willing to participate and provide informed consent were enrolled for the study. Smear negative and TB patients on anti-TB treatment were excluded from the study. A total of 459 TB presumptive patients were enrolled during the study period. Socio-demographic and clinical data were collected using the data collection form designed for purposes of the study. All missing information was collected from patient medical record. The data collection form captures patient demographic data, signs and symptoms, chest X-ray findings and empirical TB treatment initiation.

Spot-Moring-spot sputum was collected for diagnostic purposes using sterile falcon tubes then direct ZN examination was done at St. Peter's TB Specialized Hospital. Morning sputum this found three times smear negative was stored at 2-8°C for further testing and used for culture assay, TB-LAMP, Xpert MTB/RIF assay and fluorescent microscopy examination. Culture assay was done using Löwenstein-Jensen (LJ) and Mycobacterium Growth Indicator Tube (MGIT). The quality of morning sputum was evaluated and graded as M_1 (mucoid with no suspicion of pus), M_2 (predominantly mucoid, suspicion of pus), P_1 (1/3 purulent 2/3 mucoid), P_2 (2/3 purulent 1/3 mucoid), and P_3 (greater than 2/3 purulent) (Mitarai et al. 2011).

Loop-mediated isothermal amplification: TB LAMP was done on morning sputum at SPH and Ethiopian Public Health Institute (EPHI). The LAMP reaction was performed with a Loopamp DNA amplification kit (Eiken Chemical Co., Ltd., Tochigi, Japan). DNA was extracted using the Loopamp extraction kit. Sixty micro liter of sputum was added into extraction kit tube and incubated for 5 minute at 90°C. Thirty micro liter of DNA extract was then dispensed into a reaction tube and to reconstitute the dried reagent. The reaction tube was inverted several times, then the tube was incubated at 67°C for 40 min and the reaction was terminated by heating at 95°C for 2 min. The result was viewed for green color production in the presence of a LAMP amplicon, and the absence of amplification is shown by reaction tubes retaining the orange color (Kohan et al. 2011). TB-LAMP was taken as positive if either of the study sites was reported as positive.

Xpert MTB/RIF assay: Xpert MTB/RIF assay was done on morning sputum samples. At least 2ml were taken from morning sputum sample for Xpert MTB/RIF assay and the remaining sample was used for culture. Xpert MTB/RIF assay was performed in a self-contained cartridge using Xpert MTB/RIF amplification kit (Cepheid, France).Reagent was added into sputum sample in 2:1 ratio. The mixture was incubated with continuous shaking for 15 minutes at room temperature. Two milliliters of diluted sample was added into cartridge.

The cartridge was inserted in the machine. The result was interpreted by GeneXpert DX System (WHO 2013). When the result was error/invalid, retesting was done on the leftover sample. If the test remains error/invalid for the repeated GeneXpert testing, the result was taken as error/invalid.

Culture and identification: The sample was processed using NALC-NaOH method. Equal amount of NALC-NaOH was added into the sputum samples. Then the mixture was votrexed and kept for 15 minutes at room temperature. Phosphate buffer (pH =6.8) was added up to 45 ml to neutralize the NaOH and then centrifuged for 15 minutes at 3000g. After decanting the supernatant, a 2ml amount of phosphate buffer was added to reconstitute the sediment. An aliquot of 50-100µl of the sample was then inoculated into two bottles of LJ medium and 500ml to MGIT. The inoculated media were then incubated at 37°C. Growth is monitored for contamination and fast growers in the first week. Growth is followed weekly until the 8th week for LJ and 6th week for MGIT. LJ culture was considered negative when no colony was seen after 8 weeks of incubation and MGIT culture was considered negative after 6th week incubation and flagged as negative by the machine (Kantor et al. 1998). Identification of MTB complex was done by Capilia TB test on positive cultures. Testing and interpretation was done as per the manufacturer's recommendation (Abe et al. 1999). The culture result was reported as positive if *Mycobactrium* TB Complex grows on at least either of the two culture methods (MGIT and LJ). The result was taken as negative if no *Mycobactrium* TB Complex grows on both culture methods/ either of the two culture methods was negative and the other is contaminated.

Data management and Statistical analysis: Data was captured and analyzed using SPSS (IBM, version 20). Chi-square test compared the characteristics of the study population. For the predictors of empiric TB treatment initiation, Odds ratio (OR) with 95% confidence interval was used. Sensitivity, specificity, negative and positive predictive values were defined for smear microscopy, molecular detection and empirical TB treatment using the culture result as gold standard. P-value less than 0.05 were taken as statistically significant while comparing the diagnostic performance of the detection methods. The study was performed on the left over samples that were collected for diagnostic purposes. Before the patient gives the sample, he/she was informed to use the left over sample and written consent was obtained. The smear microscopy, Xpert and culture results were reported back to the physician on time for treatment purpose. Ethical clearances were obtained from EPHI and St. Peter's TB Specialized Hospital**.**

Results

Study participant characteristic: Four hundred fifty nine smear negative TB presumptive patients were enrolled in this study. Most (57%) of the study participant were female; the median age was 40 IQR (28–55) years. HIV test results were available for 41% (190/459) of the study participant and the prevalence of HIV among those individuals were 30%. Anti-TB treatment was known for 383 study participants and 46 of them were on anti-TB treatment empirically based on site smear result and other findings. Majority (68%) of the study participants were submitted good quality specimen. The background information of the study participants is summarized in Table 1.

Table 1: Background information of the study participant

Variables		N	%
Sex	Female	197	43
	Male	262	57
HIV status	Positive	57	30
	Negative	133	70
TB in close contact	Yes	33	9
	No	359	91
X-ray	Yes	340	83
	No	72	17
Treated for TB	Yes	46	12
	No	337	88
Rx for smear negatives`	Clinical only	7	16
	Clinical and X-ray	36	84
Sputum quality	M1&M2	146	32
	P1 to P3	312	68

N-Total number of participants; HIV-Human Immunodeficiency Virus; TB-Tuberculosis

Association of empirical treatment with confirmatory results and patient symptoms: Forty six smear-negative patients using direct ZN were treated empirically. The sensitivity and specificity of

empirically treated when compared with culture assay was 45.8% and 90% (Table 2). Patients who have HIV positive results [OR=4.62, 95% CI (2.30-9.31), p<0.001], chest pain [OR=2.27, 95% CI (1.32-3.90), p=0.003] and hemoptysis [OR=2.79, 95% CI (1.40-5.54), p=0.005] were significantly associated with empiric TB treatment initiation.

Table 2: Empirical treatment of the patient and its culture result

	Treated for Anti-TB	Not treated for Anti-TB	Unknown treatment status	Total
Culture Positive	11	13	6	30
MGIT	10	10	6	26
LJ	10	9	4	23
Culture Negative	34	306	67	407

Diagnostic contribution of FM microscopy and culture over direct ZN: The overall culture positivity rate was 6.8% (30/439), of which 6.6% (26/391) was by MGIT and 5.3% (23/436) was by LJ method. Direct and concentrated fluorescent microscopy adds 0.9% and 1.3% detection rate compared to direct ZN.

Performance of TB-LAMP and Xpert MTB/RIF: Participants whose cultures were contaminated (n=20), NTM (n=2), or error/invalid on Xpert MTB/RIF assay (n=21) were excluded from performance analysis. The sensitivity, specificity, PPV and NPV of TB-LAMP were compared to culture and presented in Table 3. Intra-test performance of TB-LAMP between testing sites varies significantly (p<0.001). The performance of Xpert MTB/RIF assay was also analyzed using sensitivity, specificity, PPV and NPV of TB-LAMP and shown in Table 4. The agreement of TB-LAMP and Xpert MTB/RIF assay varied significantly (kappa value 0.628).

Table 3: The sensitivity, specificity, PPV and NPV of TB-LAMP

	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Accuracy
All (HIV+, HIV- & unknown)	61.5	96.6	53.3	97.5	94.5
HIV positive	100	95.9	71.4	100	96.3
Testing sites					
SPH	50	97.5	56.5	96.8	94.6
EPHI	50	97.9	61.9	96.7	95

Table 4: The sensitivity, specificity, PPV and NPV of Xpert MTB/RIF

	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Accuracy
All (HIV+, HIV- & unknown)	70.8	97.2	60.7	98.2	95.6
HIV positive	100	97.9	83.3	100	98.1
Before retesting	69.5	97.1	61.5	97.9	95.4

Discussion

In resource limited countries, smear microscopy is the frontline diagnostic tool for smear negative TB patients. Clinicians often make decisions to initiate TB treatment based on clinical and radiological findings (Siddiqi et al. 2003). In this study, the sensitivity of empirical TB treatment compared to culture was 46% which means 54% of culture confirmed TB patients will be left untreated unless sensitive diagnostic tools are used. The present findings agree with the report from Uganda (sensitivity=38.1%) and Tanzania (sensitivity=37%) (Swai et al. 2011; Davis et al. 2010). The low sensitivity in the present finding emphasizes the need for sensitive diagnostic tools at point of care level. Conventionally, isolation of mycobacterium using culture is the most sensitive diagnostic tool. Culture can detect as few as 10–100 bacilli/ml of specimen (Parsons et al. 2011). In the present study, the diagnostic contribution of culture was 6.8% which was much lower than the findings reported by Swai et al. (2011) and Soto et al. (2013), the contribution of culture was 27% in both studies.

The lower contribution of culture may be because having patients that are not TB presumptive are being examined rather than incipient TB cases. This indicates the need for strengthening TB presumptive selection. Several algorithms and clinical scoring have been used to predict smear-negative tuberculosis based on local epidemiology. The algorithms they use provide reasonable approach for the diagnosis of smear negative TB (Swai et al. 2011; Soto et al. 2013). Those algorithms allow the efficient use of laboratory resources for patient management particularly for cost intensive diagnostic tools.

Molecular diagnostic assay have great advantages in terms of speed and sensitivity which is key for early identification of TB (Boehme et al. 2007). Previous studies reported that the sensitivity of TB LAMP for smear negative and culture positive was 48.8-55.6% (Boehme et al. 2007; Mitarai et al. 2011; Ou et al. 2014). Xpert MTB/RIF assay as an add-on test following a smear negative has 67% pooled sensitivity (95% CI 60% to 74%) (Steingart et al. 2014). In the present finding, the sensitivity of TB LAMP and Xpert MTB/RIF assay was 61.5% and 70.8% respectively. Both molecular detection methods have sensitivity close to the upper limit of the previous report. Our finding indicates that TB LAMP and Xpert MTB/RIF assay can provide a confirmatory result for at least two third of TB cases which prompt initiation of timely therapy to reduce TB-associated morbidity and mortality as well as transmission.

The yield of TB diagnostic test performance can be increased by improving the quality of sputum, moreover, proper selection of purulent part for testing is also equally important (Ho et al. 2015). Here, the performance of TB-LAMP between individuals varies significantly. This might be due to the selection of purulent part of the sputum. TB-LAMP was tested by taking 60µl from the sputum. Those diagnostic tests which take small portion of sputum might be affected by improper selection of purulent part for testing.

Conclusions

The diagnostic contribution of culture and concentrated smear was low. The low positivity rate of laboratory diagnostic methods suggests the need to strengthening better TB presumptive selection. TB-LAMP and Xpert MTB/RIF assay can provide confirmatory results for at least two third of TB cases.

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Goiter prevalence and median urinary iodine concentration in Ethiopian reproductive age women and school age children

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Abstract
Background: Iodine deficiency occurs when the diet is low in iodine. Goiter prevalence in school-age children is an important indicator of iodine deficiency disorders in a population. Urinary iodine concentration is the prime indicator of a person's recent nutritional iodine intake. The effectiveness of universal salt iodization is reflected by an increase in urinary iodine excretion in school age children.
Objective: To assess goiter rate and urinary iodine level in reproductive age women and school age children in Ethiopia.
Method: National and regional representative cross-sectional study was conducted. Data was collected from 356 enumeration areas across the country. Households with in each enumeration area were selected randomly. Goiter examination was done by trained data collectors with background of nursing, laboratory technician and nutritionists using WHO goiter categorization. Urine samples were collected from reproductive age women and school age children. Urinary iodine excretion was determined by the Sandell–Kolthoff reaction method.
Results: The prevalence of goiter was 10.8% in reproductive age women and 3.7% in school age children. Highest goiter rate was found in BenishangulGumuz (28%) and Tigray (20%) regions. There was goiter rate and median urinary iodine concentration difference between regions. Median urinary iodine concentration was 106 µg/l in school age children and 98 µg/l in reproductive age women with cumulative median urinary iodine at national level of 101.1µg/l. Half of school age children and reproductive age women were getting adequate iodine intake. Based on rapid test kit assessment, the coverage of iodized salt was around 85%, but only 26% of the household gets adequate iodized salt (>15 ppm).
Conclusion: There is promising progress to eliminate iodine deficiency disorder in Ethiopia. Adequately iodized salt intake is very limited in both age groups. Considering that the presence of both iodine deficiency and excessive intake in Ethiopia, health planners and programmers need to focus on standardizing salt iodization process and strengthening control of illegal non-iodized salt trading.
Key words: Goiter, iodine deficiency disorder, salt, urinary iodine.

Introduction
Iodine deficiency disorder (IDD) is a global public health issue (Li et al. 2010) and claimed to be the world's single most preventable cause of brain damage and mental retardation (Mannar and Dunn 1995). Iodine is needed to make thyroid hormones which are essential for normal metabolism, growth, and brain development (WHO 2007). Different environmental and individual factors are involved in the etiology of goiter, but iodine deficiency is the most important epidemiologic factor (Brauer et al. 2006). Iodine status is known to be directly related to intake (Chakraborty et al. 2010) and the sources of dietary iodine are water, food and iodized salt available in the household (Chandra et al. 2008). Iodine deficiency primarily occurs when the diet is low in iodine due to low levels in foods from plants and animals. The enlargement of the thyroid gland due to dietary iodine intake consistently lower than 50 µg/day which usually results in iodine deficiency called goiter. Goiter prevalence in school-age children is an important indicator of iodine deficiency disorders in a population (Zimmermann et al. 2004) and the concentration of iodine in the urine is the prime indicator of a person's nutritional iodine status. The effectiveness of universal salt iodization is reflected by an increase in urinary iodine excretion (Gomo et al. 1999). Iodized salt is the best means for providing iodine to iodine-deficient populations (Mannar and Dunn 1995) and has greatly reduced the prevalence of severe iodine deficiency disorders in the world. Ethiopia is a country with a 25% national goiter prevalence and high prevalence of iodine deficiency disorders which continues to affect a large number of the population (Cherinet and Kelbessa 2000) associated with low iodine intake (Tebeb 1993). Other studies have also shown iodine deficiency rate of 35.8% which

significantly associated with reproductive failure (Abuye and Berhane 2007). The problem of iodine deficiency is also very high in school children. A study conducted in ten different villages of school children measured by palpation has shown more than 53% goiter prevalence and was higher in females (Cherinet and Kelbessa 2000). Urinary iodine is the most important biochemical indicator that indicates current state of iodine nutrition and also used as a valuable indicator for the assessment of IDD because 90% body's iodine is excreted through urine (Chandra et al. 2008).

Recognizing the burden of the problem, the Government of Ethiopia implemented national program for the control of iodine deficiency disorders including, legislation decree for universal salt iodization in the country (Ethiopian Parliament 2010). However, there is a need to undertake regular cyclic surveys to assess the urinary iodine excretion and reduction of goiter problem in the country. As to the knowledge of authors of this paper, there is no recent nationally available evidence on the concentrations of urinary iodine and total goiter rate in Ethiopia. This finding provides the current status of iodine nutrition and status of universal salt iodization in the country which will helpful for health planners, program implementers and control authorities to design appropriate interventions.

Materials and Methods

A national and regional representative population based cross-sectional study was conducted. The study was conducted in 9 regions and 2 city administrations and samples were selected from 356 enumeration areas and 3805 households were visited. Two stages of sampling were applied. In the first stage, national list of enumeration areas (EAs) was obtained from the Central Statistics Agency (CSA) and used as sampling frame. The CSA randomly selected EAs using probability proportional to size and provided to survey team. At the second stage of sampling, EAs were visited by team of data collectors to conduct a quick listing of all the households within the boundary of each selected EAs. Within each EAs, households were selected randomly. All participants involved in this study were asked to their consent. All the eligible reproductive age women and school age children were clinically examined for the enlargement of thyroid (goiter) by trained data collectors using palpation method. Goiter grading was done based on WHO/UNICEF/ICCIDD criteria; Grade 0: no goiter; Grade1: palpable thyroid but not visible; Grade 2: visible thyroid with neck in normal position (WHO 2007).

Ten ml urine sample was collected from reproductive age women and school age children for urinary iodine measurement. Subjects were provided with a pre-labeled 60 ml urine collection plastic container and asked to provide urine sample. Upon receiving the urine sample, the team ensured the screw cap was fastened and the tube was dry. Urine tube was placed in ice box and transported to the nearest health facility at the end of each day. Upon arrival at the health facility, the laboratory technologist counter checked the sample label with the laboratory control checklist. The urine samples were then transferred into two same label 5ml cryovial tubes and each set of urine samples were placed into a separate cryovial box and frozen at -20^{0}C until shipment to the EPHI.

A onetime thawing of the urine samples was conducted and analysis of urinary iodine was done at the Food Science and Nutrition Laboratory of EPHI. The urinary iodine test was done based on the Sandel Kolthoff reaction method which utilizes the catalytic effect of iodide on the oxidation reduction of Ceric ions (Ce^{4+}) and Arsenite (As^{3+}) accompanied by color change. The speed of the color disappearance is proportional to the concentration of iodide catalyzing the reaction. To quantify the iodine content in salt samples, about 20 g of salt from each household was collected and the salt samples were kept at room temperature in the laboratory until analysis. Sample of salt collected from each household was measured for iodine level using the iodometric titration method.

Results

Of the total listed individuals from all households, nearly 26% were school age children. Sex composition was almost similar in all age groups. A total of 2155 reproductive age women and 2156 school age children were assessed for goiter (Table 1).

Table1: Percentage distribution by age and sex of the study participants

Sex	N	<5 years	6-14 years	15-49 years	>49 years
Male	8216	18.7	26.2	44.1	11.1
Female	8652	17.0	25.5	46.7	10.8
Total	16868	17.8	25.8	45.4	10.9

Goiter rate among reproductive age women: National goiter rate was 10.8% in reproductive age women. Highest goiter rate was found in Benishangul Gumuz region (28%). Tigray region has the highest visible goiter rate (11%). Lowest prevalence of all forms of goiter was examined in Afar region. There was no goiter case examined in Somali region among reproductive age women (Figure 1).

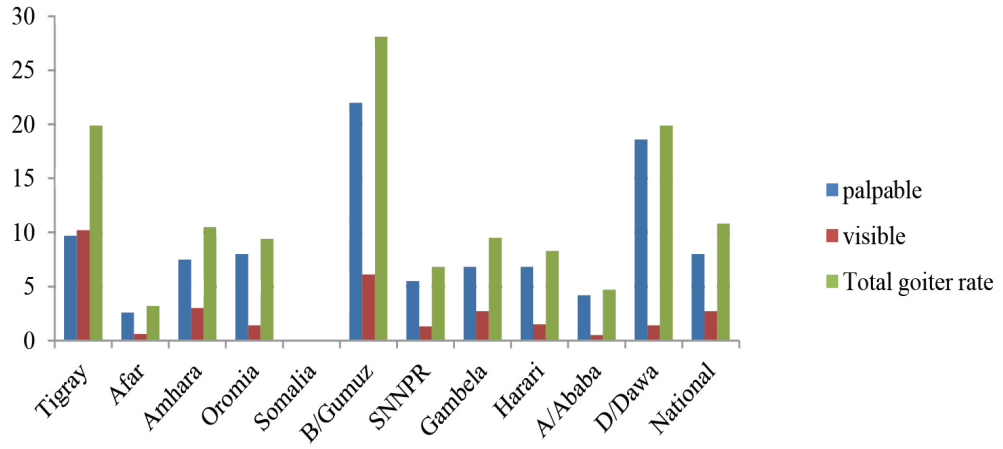

Figure1: Prevalence of goiter rate among reproductive age women, Ethiopia, 2015

Goiter rate among school age children: Nationally, school age children total goiter was found to be 3.7% with highest rate in DireDawa (14.1%) and BenishangulGumuz (11.4%). Except for Oromia, SNNPR and Gambela regions, total goiter rate in school age children was above the national level. Visible and palpable goiter cases were not found in Addis Ababa, Afar and Somali regions among school age children (Figure 2).

Median urinary iodine concentration: Nearly half of the reproductive age women and 54.2% of school age children had adequate iodine intake at the national level.

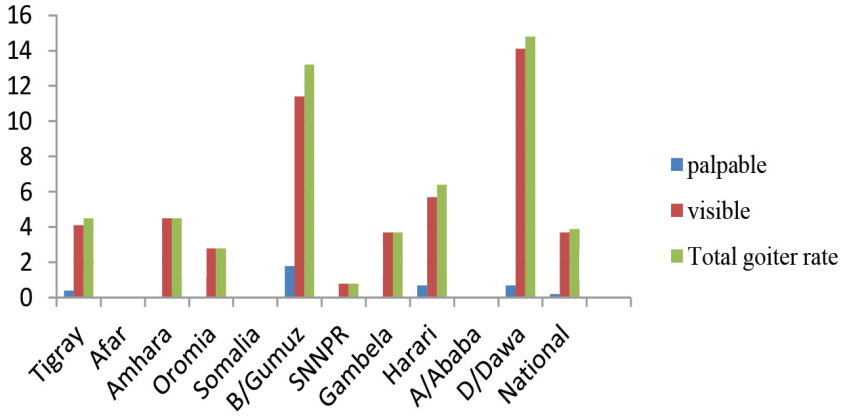

Figure2: Prevalence of goiter rate in school age children, Ethiopia, 2015

Proportion of urine samples with adequate iodine concentration from reproductive age women of Somali, Afar and Dire Dawa regions were 88, 86 and 73 µg/l, respectively with national median urinary iodine concentration of was 98.18 µg/l. The highest and lowest concentrations were observed in Afar and Amhara regions, respectively (Table 2). The median urinary iodine concentration in school age was 105.59 µg/l. The highest median urinary iodine was measured in Afar, Somali and Tigray regions respectively and lowest intake of iodine was measured in Amhara, Oromia and SNNPR region (Table 3).

Table 2: Median urinary iodine concentration of reproductive age women, Ethiopia, 2015

Region	n	Median UIC	Urinary iodine concentration % urinary Iodine concentration WHO Cutoff points			
		µg/l	<20 µg/dl	20-49 µg/dl	50-99 µg/dl	>100µg/dl
Tigray	219	82.51	7.8	19.6	31.1	41.6
Afar	81	217.09	2.5	7.4	3.7	86.4
Amhara	227	68.26	4.8	26	39.2	30
Oromia	225	105.63	5.3	11.1	30.7	52.9
Somali	110	208.22	0	1.8	10	88.2
B/Gumuz	124	73.56	1.6	21.8	43.5	33.1
SNNPR	180	112.36	0.6	14.4	31.1	53.9
Gambela	119	69.6	2.5	25.2	36.2	36.1
Harar	210	89.3	3.8	19.5	34.8	41.9
Dire-Dawa	136	139.92	1.5	4.4	20.6	73.5
Addis Ababa	182	95.16	2.2	8.8	42.8	46.2
National	1813	98.28	3.4	15.	31.5	49.5

Table 3: Median urinary iodine concentration of school age children, Ethiopia, 2015

Region	N	<15 ppm Iodine	≥15 ppm Iodine
Tigray	344	44.8	55.2
Afar	193	83.4	16.6
Amhara	447	85	15
Oromia	382	76.7	23.3
Somali	257	50.6	49.4
B/Gumuz	240	79.6	20.4
SNNPR	300	86.3	13.7
Gambela	137	90.5	9.5
Harar	298	77.2	22.8
Dire-Dawa	244	70.9	29.1
Addis Ababa	379	78.1	21.9
National	3221	74.2	25.8

Household iodized salt coverage in Ethiopia: From 3200 households, only 26% of them were getting more than 15 ppm iodine in salt. The highest coverage of adequately iodized salt was found in Tigray, Somali, Dire Dawa and Oromia regions, respectively while there is lowest coverage in Gambela and SNNPR regions. High variability in proportion of households with salt iodine content was found across the country.

Table 4: Household iodized salt coverage in by region

Urinary Iodine Concentration						
Region	n	Median UIC	% Urinary iodine concentration WHO Cutoff points			
		µg/l	<20 µg/l	20-49 µg/l	50-99 µg/l	>100µg/l
Tigray	239	206.58	1.3	2.9	17.1	78.7
Afar	124	339.79	0.7	2.2	5.2	91.9
Amhara	229	72.14	3	27.1	40.6	29.3
Oromia	262	80.81	0.8	19.1	43.1	37
Somali	109	333.19	0	2.8	6.4	90.8
B/Gumuz	111	85.58	2.7	15.3	48.6	33.3
SNNPR	175	80.34	14.9	16	29.1	40
Gambela	101	103.28	5	14.9	26.6	53.5
Harar	263	95.97	1.1	13.3	37.6	47.9
Dire Dawa	123	140.73	0	4.1	20.3	75.6
Addis Ababa	116	94.33	0.9	13.8	39.7	45.7
National	1852	105.59	2.7	12.9	30.2	54.2

Discussion

Ethiopia is a country with a high prevalence of iodine deficiency disorder which continues to affect a large number of a country`s population. In this study, goiter rate was found 10.8 % in reproductive age women, which is a major public health concern based on classification of WHO/UNICEF/ ICCIDD. Prevalence of goiter in school age children of Ethiopia was high and varied among regions (Abuye and Berhane 2007; Kidane and Woldegebriel 2006). This study showed less than 5% total goiter rate in school age children which is not considered as a public health problem but similar to past studies varies across the regions (Tebeb 1993). This change may be attributed to the mandatory fortification program done in the country which is similar to the success of South Asia countries by large scale IDD control programs (UNICEF 2002). Quantification of urinary iodine is accepted as a valuable estimation of the recent iodine intake (Als et al. 2003). In Ethiopia there is no evidence on median urinary iodine concentration for reproductive age women at national level but the median urinary iodine for children was reported 24µg/l (Cherinet and Kelbessa 2000). In this study, the median urinary iodine in reproductive age women and school children was found 98µg/l and106µg/l respectively. This value is close to the recommended for reproductive age women and within the recommended range for children. We have also found big variation in urinary iodine concentration among regions. The difference might be due to homogeneity problem during salt fortification and instability of iodine in the salt.

Conclusion

The problem of iodine deficiency disorder is decreasing in reproductive age women and school age children. Goiter rate reduction is promising to make iodine deficiency as no more public health problem in Ethiopia. To maintain the current progress, health planners and programmers needs to focus on standardizing salt fortification process and strengthening control of trading system in Ethiopia.

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Anti-Retro Viral Treatment implementation and its barrier at St. Paul's hospital millennium medical college

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Abstract
Background: HIV infection is the major public health, social and economical problem around the world. However, retain patients in care and adherence to antiretroviral therapy standards are the major problems, Ethiopia has an estimated 298,512 adults on ART.
Objective: To study ART implementation and its barriers at St. Paul's hospital millennium medical college.
Methods: By using cross-sectional study, 299 clinical records of adults were randomly retrieved from Sept 11, 2013, to Sept 10, 2014, and an in-depth interview was done on 9 key informants. SPSS version 20 and thematic analysis were used for the quantitative data and qualitative data analysis. The associations were measured by odds ratio along with its 95% confidence interval.
Results: About 64.9% (194) of clients were missing important components of HIV care recommended by the national guidelines. There is no resource problem regarding ART; implementation guideline, laboratory facilities and drugs were available. However, there is a high workload due to the shortage of human resource.
Conclusion: Adherence to standards of HIV/AIDS care at the facility was inadequate. Compliance was better at the start of ART but declined during the follow-up period. Regular assessment of ART implementation with the national guideline can optimize the effectiveness of national ART programs.
Keywords: HIV, National Guideline, ART, Adherence, Indicator

Introduction
Globally, out of 36.9 million people living with HIV, 34.3million were adults. There were 2.0 million new HIV infections and 1.2 million AIDS deaths (UNAIDS 2014). Even though ART decreased morbidity and mortality(Egger et al. 1997; Vittinghoff et al. 1999; Assefa et al. 2012), only 9.7 million low-and-middle-income countries PLWHIV were receiving ART of as compared to 10.6 million PLWHIV live in high-income countries (UNAIDS 2013; UNAIDS 2014; Braitstein et al. 2006; Assefa et al. 2005-2007). Among 793,700 PLWHIV in Ethiopia, 298,512 adults were on treatment and 70.3% of individuals who ever started ART were currently on treatment (Country progress reports on the HIV response 2014; Multisectoral HIV/AIDS Response Annual Report 2013). According to Ethiopian Demographic Health Survey (EDHS), prevalence of HIV in adults was 1.5% (Central Statistical Agency (CSA) & ICF International 2012).

One of the priority areas indicated in the national guideline is monitoring the number and percentage of different group of populations who have initiated ART based on the new eligibility criteria (FMoH 2014). Although many hospitals and health centers are providing ART services, there were limited studies done on its implementation as compared to the national guidelines. Therefore, this study was aimed to assess ART implementation and its challenges which may provide information for future programming.

Materials and Methods
Facility based cross-sectional study was done on 299 randomly selected records of ≥15 years old clients those enrolled on ART from September 2013 to September 2014 and on 9 full-time staff key informants. Patients who have been in treatment for at least 6 months were eligible for record review and patients who transferred in or out to other health facilities, lost follow up, dead, pregnant women and breast feeding women with in the study period were excluded from the study. National and WHO ART implementation guideline formats for data extraction, semi-structured questionnaire of United Nations Program on HIV/AIDS (USAIDS), equity and access to ART in Ethiopia for the in-depth interview were used (Braitstein et al. 2006; WHO 2004; (Egger et al.1997; Vittinghoff et al.1999; Assefa et al. 2012).. Descriptive statistics were used to determine the magnitude of adherence to the national standard guideline. Qualitative data were analyzed by using bivariate and multivariable logistic regressions.

Ethical clearance was obtained from Mekele University, Addis Continental Institute of Public Health and SPHMMC.

Results

About 59% were females' record and 44.1% were clients within the age range of 26-36 years. About 53.3%, 37% and 52.5% were married, had primary education and full time employees respectively. Seventy seven percent of the retrieved data belonged to orthodox Christian followers. About 78.3%, 72.9%, 79.3% and 81.3% of clients were never use tobacco, alcohol, soft and hard drugs respectively. Even though all the 299 patients were eligible for CPT, for only 75.6% of them were prescribed (Table1). About 64.9% of clients were not adhere the 8 standard indicators as per the national guideline. The in-depth interview with the key informants showed presence of Cotrimoxazole in the stock.

Table 1: Status of retrieved client charts based on indicators at St Paul Hospital Millennium Medical College, September 2013 – September 2014

Indicators	Category	
	Yes Number (%)	No Number (%)
CD4 count or WHO clinical stage at baseline	299 (100)	-
Prescribed daily Cotrimoxazole	226 (75.6)	73 (24.4)
Assessed for TB in every visit	262 (87.6)	37 (12.4)
Adherence counseling in every visit	29799.3	2 (0.7)
CD4 count measured at least once with in the last 6 months	186 (62.2)	113 (37.6)
Started ART within 2 weeks after eligibility is confirmed	292 (97.3)	8 (2.7)
Laboratory workup before initiating ART	277 (92.6)	22 (7.4)
Currently on ART	275 (92.0)	24 (8.0)

Table 2: Factors associated with patients to be currently on ART at SPHMMC from Sept. 2013 – Sept. 2014

Characteristics		Yes (N)	No (N)	COR (95% CI)	P-value	AOR(95% CI)	P-value
Sex	Male	110	12	-	-		
	Female	165	12	-	-		
Age	15-25	39	4	0.94(0.29-3.05)	0.91	1.79(0.31-10.4)	0.51
	26-36	119	13	1.68(0.43-6.6)	0.46	1.91(0.41-8.87)	0.40
	37-47	82	5	1.79(0.31-10.4)	0.51	1.07(0.19-5.76)	0.94
	48+	35	2	1			
Education	No education	56	3	1.57(0.41-5.92)	0.50	0.64(0.17-2.4)	0.51
	Primary	100	11	0.76(0.31-1.87)	0.56	1.3(0.53-3.2)	0.56
	Secondary/Tertiary	119	10	1			
Tobacco	Never	220	14	1			
	Rarely	19	3	0.32(0.124-0.86)	0.024*	0.38(0.14-0.98)	0.78
	Never	207	11	1			
Alcohol	Rarely	30	6	0.28(0.10-0.79)	0.016*	0.92(0.28-3.03)	0.89
Soft drugs	Never	222	15	1			
	Rarely	14	2	0.38(0.14-0.98)	0.046*	1.25(0.23-6.78)	0.79
Hard drugs	Never	228	15	1			
	Rarely	6	1	0.34(0.13-0.84)	0.021*	1.17(0.12-11)	0.89

COR: Crude Odds Ratio *AOR: Adjusted Odds Ratio* **P-value<0.05*

In the bivariate analysis, tobacco, alcohol, soft drug and hard drug usage were significantly associated with patients` eligibility for ART unlike in the multivariate logistic regression in which no factor was independently associated. According to the in-depth interview, changing of clients' addresses, low economic status of clients, lack of recognition of ART case team as outpatient departments by the hospital officials, lack of conducive environment, lack of ventilation and being narrow of the examination rooms were the challenges to implement the guideline and to adhere ART.

Table 3: Factors associated with patients started ART within 2 weeks of eligibility at SPHMMC from Sept. 2013 to Sept. 2014

		Yes in №	No in №	COR (95% CI)	P-value	AOR(95% CI)	p-value
Sex	Male	120	2	-			
	Female	171	6	-			
Age	15-25	42	1	1.17(0.07-19.3)	0.91	0.86(0.05-14.1)	0.91
	26-36	127	5	0.70(0.08-6.23)	0.75	1.41(0.16-12.5)	0.75
	37-47	86	1	2.39(0.14-39.2)	0.54	0.41(0.25-6.87)	0.54
	48+	36	1	1		1	
Education	No education	56	3	1.43(0.31-6.62)	0.64	6.8(0.69-67.3)	0.09
	Primary	107	4	6.8(0.69-67.3)	0.09	4.8(0.52-43)	0.16
	Secondary/Tertiary	128	1	1		1	
Tobacco	Never	229	5	1		1	
	Rarely	21	1	0.44(0.08-2.38)	0.34	1.02(0.08-11.9)	0.99
	Never	213	5	1		1	
Alcohol	Rarely	35	1	0.50(0.09-2.68)	0.42	1.62(0.14-18.7)	0.69
Soft drugs	Never	232	5	1		1	
	Rarely	15	1	0.47(0.08-2.52)	0.38	0.68(0.05-8.0)	0.76
Hard drugs	Never	238	5	1			
	Rarely	7	0	-	-	-	-

Discussion

In this study, prevalence of patients' eligibility for ART was 92%. A study conducted in Mizan-Teferi, Ethiopia recommended the need to understand the health-seeking behavior of patients for ART to implement strategies in HIV clinics (Moshago et al. 1995). Adherence to ART and CPT were 75.6% which is lower than studies done in northern Ethiopia (97.3%) (Kiflie et al. 2009), in Jimma University Specialized Hospital (Diriba et al. 2008) and in Asia-Pacific, sub-Saharan Africa and Americas (88%) (Stephany et al. 2014). This could be due to failure to prescribe CPT for eligible patients during clinic visits and poor compliance of patients with CPT accordingly with the national guideline even though there is no shortage of Cotrimoxazole in the stoke. But increase adherence to CPT prevents the occurrence of fatal opportunistic infections (Grimwde et al. 2005) and Abidjan (Anglaret et al. 1999) if it is used according to WHO`s and UNAIDS recommendation (WHO, UNAIDS 2000).

Unlike the baseline (100%), determination of clients' CD4 or WHO clinical stage at least once CD4 count measured during the last 6 months only was lows (62.2%) which was similar to a study conducted in northern Ethiopia (Kiflie et al. 2009). Similarly, need to initiate ART within 2 weeks clinical eligibility (97.3%) and basic laboratory workup before initiating ART (92.6%) were higher than the same activities done in follow-up care. This finding is comparable to another study done in Ethiopia (Kiflie et al. 2009), Uganda (Burua et al. 2014), USA (McGlynn et al. 2003), which showed that patients are more likely to receive the recommended HIV care during their first visit. In this study, 87.6% of HIV clients were checked for TB in each visit. This finding was the same as a study conducted in Uganda (Burua et al. 2014).

Conclusion and Recommendation

In this study, seven out of eight standard indicators were not adhere as per the national guide. Along with physicians, other health care providers and higher hospital officials should play their role to improve health-seeking behavior of patients for ART and the implementation status of guidelines. Moreover, there is a need of supportive supervision which can ensure and improve quality, effectiveness, and efficiency of services provided.

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Plasma HIV RNA level and its effect on transmission to infants among pregnant mothers on ART/prophylaxis

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Abstract
Background: Antiretroviral drugs, during and after pregnancy can reduce the risk of HIV transmission from pregnant women to their infants. This study was aimed to assess the association of plasma HIV RNA level and risk of transmission among pregnant women who are on Antiretroviral Therapy or prophylaxis.
Objective: To assess the association between the level of plasma HIV RNA and risk of transmission to their infants among pregnant women who were on ART or prophylaxis.
Methods: Facility based prospective study was conducted among 3rd trimester pregnant mothers who were on ART or prophylaxis from November, 2012 to November, 2013. Interview was done using a structured questionnaire and blood sample was collected for laboratory analysis and HIV DNA PCR was tested for the early infant diagnosis using the Amplicor HIV-1 DNA test. Additionally, retrospective data on CD4 counts and hematology were collected. HIV-1 viral load copies/ml was determined by quantifying the amount of HIV-1 RNA in plasma sample using m2000rt Real Time HIV-1 assay. Stata version 11 was used for data analysis. Statistical significance considered $P<0.005$.
Results: Twenty five pregnant mothers who were on prophylaxis and 25 who were on ART with their corresponding 48 infants were enrolled in this study. The mean ages for pregnant women on ART and on prophylaxis were 28.5± 3 years and 26.8±5.0 years, respectively. Among the ART group, 7(28%) of them had detectable viral load (mean viral load was 19,343.8 copies/ml). In the prophylaxis group, 21(84%) of them had detectable viral load (mean: 197,120 copies/ml). The mean CD4 T cells count was higher on individuals who were on prophylaxis (mean: 598.6 cells/µl) compared to those on ART (324.4 cells/µl) ($p<0.001$). The rate of protection for the mothers on prophylaxis was 92 % compared to 100 % for those on ART.
Conclusion: This finding confirms ART reduce significantly the risk of MTCT of HIV and will benefit the health of their future pregnancy outcomes by suppressing their viral load compared to those who were on prophylaxis.
Key words: ARV prophylaxis, ART, viral load, infants, transmission

Introduction
Globally, Human Immunodeficiency Virus (HIV) has become one of the world's most serious health and developmental challenge (UNAIDS 2013). Since the onset of the HIV/AIDS pandemic, currently there are 37 million people living with HIV (PLWHIV) and tens of millions of people died worldwide (UNAIDS 2016). Sub-Saharan Africa accounts more than two-thirds of the persons infected with HIV (UNAIDS/ WHO 2016). Women represent half of PLWHIV and showed HIV as the leading cause of death among women of reproductive age; globally, 3.2 million are living with the virus in which 240.000 are new infections and 190,000 of them died from AIDS (UNAIDS/WHO 2014). The use of antiretroviral drugs during and after pregnancy is a proven intervention to virtually eliminate the risk of HIV transmission to infants, as evidenced in high-income countries where new childhood HIV infections are now almost non-existent (Townsend 2008). The prevention of mother-to-child HIV transmission (PMTCT) recommendations has two key approaches: to life-long ART for HIV-infected pregnant women in need of treatment and prophylaxis or the short-term provision of ARVs (WHO 2010).

In Ethiopia, there has been an increase in the percentage of women tested for HIV. By 2010-2011, the number of HIV-positive pregnant women was 20,755 and of these, only 8,365(40.3%) received antiretroviral drugs (FHAPCO 2011); still Ethiopia is one of the 22 priority countries for eliminating MTCT of HIV (UNICEF 2011). Being high viral load of the mother at birth increases the chance of transmission similar to new infection or with advanced disease stage (Expert Panel Report and recommendations to the U.S. Congress and U.S. Global AIDS Coordinator 2010). To date, the level of MTCT of HIV based on the level of plasma viral load has not been well documented in Ethiopia. Therefore this study was aimed to assess the association between the level of viral suppression and HIV transmission from pregnant women on ART or prophylaxis to their infants.

Materials and Methods

A prospective study was conducted at selected health facilities [Saint Paul's Hospital Millennium Medical College (SP'SMMC), Yekatit 12 General Hospital, Zewditu Memorial General Hospital, Semen Health Center, Kazanchise Health Center, Woreda 6 Health Center and Woreda 7 Health Center] in Addis Ababa, Ethiopia from November 2012 to November 2013. In addition, retrospective data from all 3rd trimester HIV positive pregnant women sent to the laboratory for viral load, CD4, hematology tests during the study period and from all their HIV exposed infants under 6 weeks of age were collected. Though the sample size was calculated to be 179 for prophylaxis and 167 for mothers on ART, only 25 mothers for each group (at the prevalence rate of 3.7% and 4% respectively) with their corresponding 25 exposed infant were enrolled. Plasma samples for viral load and DBS samples for EID tests were collected from mothers and infants as part of the routine patient care in the PMTCT program. About 5 ml blood specimen was collected by venipuncture into EDTA vacutainer tubes by qualified laboratory technologist and transported to Ethiopian Public Health Institute (EPHI) laboratory within 4 hours of collection, then centrifuged at 2,000 rpm for 5 minutes to separate plasma. The plasma was stored at -80°C until testing. Dried blood spot (DBS) samples were collected from infants and stored at room temperature for DNA - PCR test. The specimens were processed for DNA extraction, amplification, detection and viral load determination (Using Abbottm2000rt Real T*ime* HIV-1 assay, Abbott Laboratories, Abbott Park, IL from200µl plasma) (Geelen 2003; Swanson 2006a). HIV-1 DNA test version 1.5 (v1.5, DNA – PCR technology) was used for the early infant diagnosis (EID) (Guyer 1998). Data was analyzed using version 11 STATA. Univariate analysis and Chai -Square test was used. A p - value < 0.05 was considered as statistically significant. Ethically approval was obtained from Aklilu Lemma Institute of Pathobiology and Saint Paul Hospital Millennium Medical College Ethical Review Board.

Results

Twenty five pregnant mothers who were on prophylaxis and 25 who were on ART with their corresponding 48 infants [25 born from mothers on ART and 23 born from mothers on prophylaxis] were enrolled in this study. All mothers delivered through normal vaginal delivery. Mean age of participants was 28.5 ±3.8 years for the ART group and 26.8±5.0 years for the Prophylaxis group (Table 1).

Table 1: The demographic characteristics and laboratory results of mothers involved in the study

Group	detectable viral load	Undetectable viral load	CD4 (cells/µl)	Age(years, Mean ±SD)	Hgb(g/dl, Mean ±SD)
On ART (n=25)	7 (28%)	18 (72 %)	324± 187	28.5± 3	12.1± 1.3
On Prophylaxis (n=25)	21 (84%)	4 (16%)	594± 179	26±5	12.8±1.1
	P<0.001(X^2 test)		P< 0.001	NS (p=0.18)	NS (p=0.07)

Two out of twenty five (8%) infants born to women on prophylaxis were died. There was no death observed in infants from women on ART. There were 5 classes of ARVs used for treatment in the ART group; 1a (4%), 1c (56%), 1d (4%), 1e (16%) and 1f (20%). All were in the first line regimen. However, all of those on prophylaxis groups were on AZT. CD4 T cell counts were measured after an average of 16.5 months, for those on ART and 2.5 months for those on prophylaxis. The mean CD4 T cells counts was higher in those prophylaxis group (mean: 598.6 cells/µl) compared to those on ART (324.4 cells/µl) (p<0.001) (Figure1).

Table 2: Classes of ARVs used for treatment in the ART group

Regimen	Drug combination
1a = d4T + 3TC + NVP	d4T - Stavudine, 3TC-Lamivudine, NVP –Nevirapine,
1c = AZT+ 3TC +NVP	AZT– Zidovudine, 3TC - Lamivudine NVP –Nevirapine
1d = AZT +3TC +EFV	AZT– Zidovudine, 3TC -Lamivudine EFV – Efavirenz
1e = TDF +3TC + EFV	TDF - Tenofovir,3TC-Lamivudine EFV– Efavirenz
1f = TDF + 3TC+ NVP	TDF - Tenofovir,3TC–Lamivudine, NVP –Nevirapine

Viral load testing was done after an average 17.9 months of their ART enrollment for the ART group and 4.8 months for the prophylaxis group. Of those ART group 7(28%) had detectable viral load with mean viral load count of 19,343.8 copies/ml (IQR: 837-20,606, and range from 345 to 83,140 copies/ml) (Figure 2).

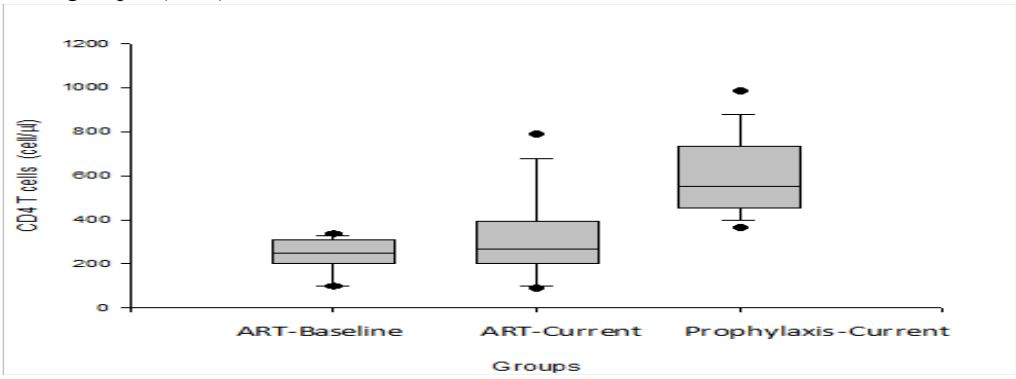

Figure 1: CD4 + T cell counts of clients at baseline for those on ART, Current on ART and on Prophylaxis

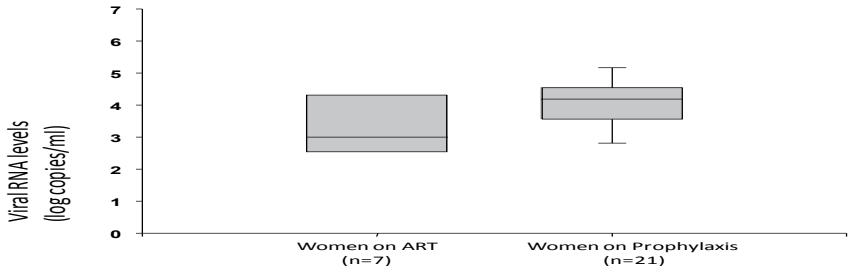

Figure 2. The level of viral RNA level among those with detectable viral load in women on ART and Prophylaxis

Among the prophylaxis group, 21(84%) of them had detectable viral load with mean viral load 197,120 copies/ml [IQR: 4,303-30,825, and range from 167 to 3,575,889 copies/ml] (Table-1). The viral load level of ART group was not significantly different (P=0.29) from those on prophylaxis, but the CD4 T cells were significantly different at the time of delivery (Figure 3).

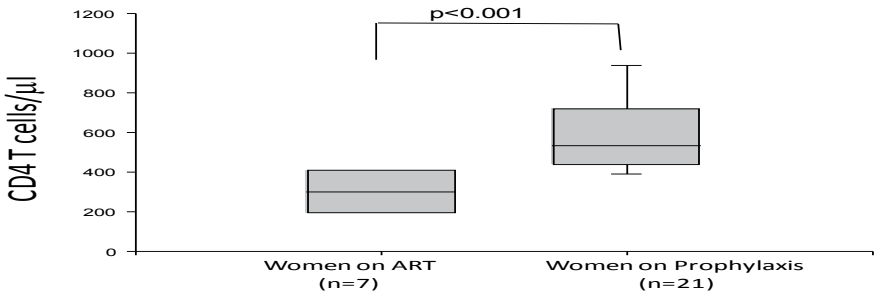

Figure 3: CD4 + T cell counts among those with detectable plasma viral load

Among those on ART, the hemoglobin level was 12.1 g/dl, median: 12.1), while those who were on prophylaxis had 12.9g/dl Median: 13g/dl). Taking the normal range values for adult women as 12 to 16 g/dl, 25% of the ART cases and 10% of those on prophylaxis had below the normal range. All infants born from mothers on ART were healthy while there were 1 still birth and 1 neonatal death born from ARV prophylaxis mothers group. The still birth was from a mother with a viral load of 3,575,889 copies/ml, CD4 count of 400 cells/µl and, Hgb level of 13.1g/dl; while the neonatal death was from a mother with viral load 92,626 copies/ml, CD4 count was 723 cells/µl, and Hgb 11.5g/dl. Except these two, all other delivered infants were HIV DNA PCR negative.

Discussion

This study showed all mothers who were on ART delivered non-infected infants, while there were 1 still birth and 1 neonatal death from those on prophylaxis; the protection rate of ARV for the mothers on prophylaxis was 92% compared to 100% for those among on ART. A study conducted in Malawi by Maria et al. (2013) showed ART was more protective than any other prophylaxis regimen. Furthermore, starting ART at least 14 weeks prior to delivery (early ART) yielded the most benefit with no transmissions noted which is consistent with WHO guideline B+ and other studies (Warszawski et al. 2008; Patel et al. 2007; Mayaux et al. 1997) those indicate complete viral suppression after 12 to 16 weeks of HAART. Moreover, a study conducted in Ethiopia by Derebe et al. (2014) showed early initiation of ART to HIV infected pregnant women contribute to the elimination of transmission to the new born babies. Furthermore, a study done by Berhan et al. (2014) showed mothers on ART for duration of less than 4 weeks were more likely to have transmission to their infants that might be due to absence of viral load suppression. This was in line with the current study that the death of two infants among the ARV prophylaxis group may suggest the fact ART is more protective than prophylaxis. In line with this, the study conducted in Kenya by Okonji et al. (2012) showed high baseline viral load and short exposure to ARVs for PMTCT are risk factors for failing to achieve undetectable viral load. These findings support the current study and new WHO guidelines B+ which recommends ART initiation for all HIV positive pregnant mothers regardless of their CD4 for maximal reduction of maternal viral load.

A study done in Benin by Denoeud-Ndam et al. (2013) provides more evidence to initiate ART before the last trimester of pregnancy to achieve undetectable plasma viral load before delivery. Early initiation, together with a high antenatal care attendance, led to high rate of virologic control. This showed ART initiation before the third trimester of pregnancy is a good predictor of virologic suppression to minimize the risk of pre and intra-partum MTCT. This recommendation can be supported by the current study, as most of the pregnant women in the ART group with longer period of treatment had undetectable plasma viral RNA level (72%) compared to those on prophylaxis (16%) with short period of prophylaxis.

A study done by Townsend et al. (2008) also suggests that, the use of ART drugs during and after pregnancy is a proven intervention to virtually eliminate the risk of HIV transmission to infants. Different studies done in different countries (Townsend et al. 2014; Rundare et al. 2012; European Collaborative Study et al. 2007; Marazzi et al. 2010; Chibwesha et al. 2011; Izzo et al. 2011 and UNAIDS 2011) showed administering combined ART and initiating early will drop MTCT of HIV four fold, which is in line with this study as most of the pregnant women in ART group were on combined therapies unlike the prophylaxis group with ZDV monotherapy which is similar to this study finding that the longer the ART taken, the higher the probability to achieve virologic suppression at the end of pregnancy.

Conclusion

This finding showed that ART suppress viral load thereby significantly reduce the risk of MTCT of HIV and will benefit for many HIV- positive pregnant women and their future pregnancy outcomes by improving maternal health compared to those who were on prophylaxis.

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Trends of research on traditional medicinal plants in Ethiopia: A preliminary review

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Abstract
Background: In Ethiopia, there are more than 6,500 plant species of which an estimated 12% are endemic and about 887 species are used as medicinal plants. Considerable efforts have been exerted on research and development of medicinal plant products in the country. However, the research trend is largely unknown.
Objective: To examine and update the trends of research and development efforts on Ethiopian traditional medicinal plants.
Methods: Data for this review were generated from research findings on Ethiopian traditional medicinal plants that appeared indifferent peer reviewed journals and proceedings during the last 30years (1985-2014) using search words of Ethiopian medicinal plants, traditional medicine, formulation, toxicity, standardization, and phytochemistry. Descriptive analysis was conducted using excel spreadsheets and the number of research findings were categorized in each disease category.
Results: A total of 454 abstracts were published in peer reviewed journals and proceedings. The most researched areas were the antimicrobial drugs, ethnomedicinal, antprotozoal phytochemistry and Knowledge, Attitude and Practice about traditional medicine study. The study also revealed that much research is yet to be done in other areas such as formulation, anticancer, diabetics, hypertension and mental disorder. Over 170 medicinal plants were screened for biological activity of which *Moringa stenopetala, Glinus lotoides, Lippia adoensis, Phytolacca dodecandra, Albizia gummifera, Artemisia afra, Croton macrostachys, Rumex abyssinicus* were the most researched medicinal plants for their phytochemistry, and pharmacological activity.
Conclusions: This preliminary review set out the trends of medicinal plant research in Ethiopia. The research and development should be intensified in the least-researched areas. It is recommended that medicinal plant research and development should be strengthened and linked to manufacturing investment if Ethiopia intends to meet the health sustainable development goals.
Key words: Research, development, commercialization, natural medicinal products, Ethiopian traditional medicine

Introduction
Plants have been used as medicinal sources since ancient times, and even today plant based pharmacopoeias continue to play an essential role in world health care. The use of biological resources for various therapies has been documented in different parts of the world, especially in East Asian countries where traditional medicines provide an alternative to modern health care system. This perhaps may be sequel to the inability of modern medicine to comprehensively address most disease conditions plaguing humankind (Kang and Phipps 2003).

The World Health Organization (WHO) estimated that 80% of the world population relies on traditional medicine prepared mainly by using natural products (animals and plants) to meet their daily health requirements (Aschwanden 2001). WHO estimates that 35, 000 to70, 000 species of plants are being used for medicinal purposes around the world (Demissew and Dagne 2001). Herbal medicine refers to medicine or drug made from plants and it is synonyms with phytomedicine, plant medicine, green medicine, traditional remedy, plant drug and forest health product among others (Elujoba et al. 2005). The WHO also defined herbal medicine as finished and labeled medicinal product that contains active ingredient(s) of aerial or underground parts of plants or combinations thereof whether in crude juices, gums and fatty oils and other substances of this natural origin (WHO 1996).

Ethiopia is endowed with diverse flora and fauna. The country has more than 6,500 plant species out of which an estimated 12% are endemic and about 887 (13.6%) species are used for medicine. The majority (80%) of Ethiopian people depends on traditional medicine for their health care, and more than 95% of traditional medicinal preparations are herbal origin (Giday et al. 2003; Demissew and Dagne 2001). Ethiopia is also home for many languages, cultures and beliefs that have in turn contributed to the high diversity of traditional knowledge and practice of the people (Abebe 1996).

Far East Asian countries such as China, South Korea

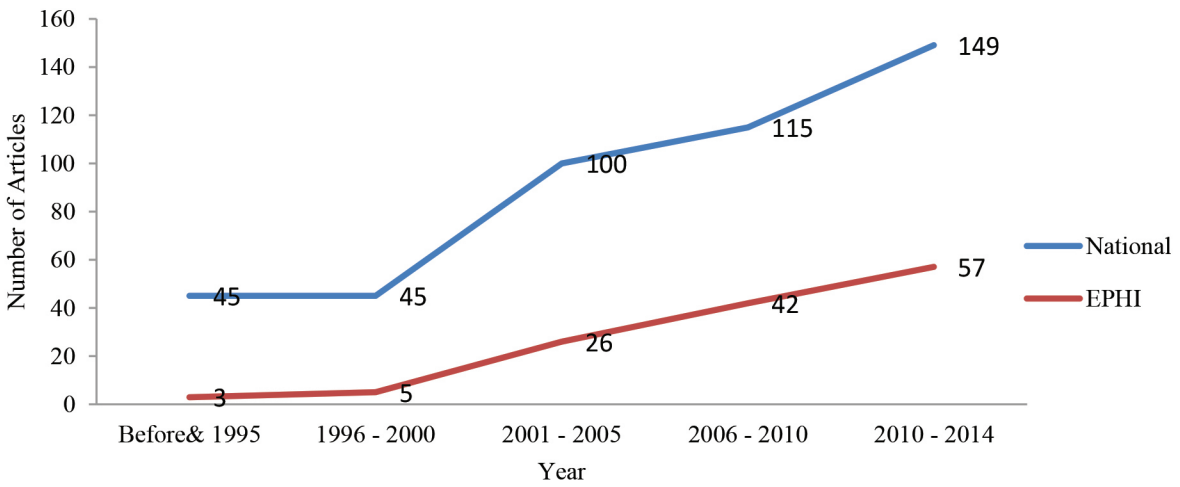
and India have been able to meet 75% of their health care needs through research, development and utilization of herbal medicines, traditional medicine practices and creating high market demand for their herbal products. The output of Chinese materia-medica was estimated to value US$83.1 billion in 2012; Republic of Korea, US$7.4 billion in 2009; United States of America, US$14.8 billion in 2008 (Nahin et al. 2009; WHO 2013). Hence, developing countries should invigorate their efforts to develop and utilize local medicines that are most appropriate to their local circumstances especially for use in the Primary Health Care and cut down huge cost associated with incessant drug importation (WHO 1996). In Ethiopia, there are efforts on research and development of herbal medicines conducted by universities and research institutions. However, its trend is not as such recognized. Therefore, the aim of this review was to chronicle and update the trend of research results in herbal medicine.

Materials and Methods

Data for this review were generated from the published research findings on Ethiopian traditional medicinal plants from different peer reviewed journals and proceedings, which were published during the past 30 years (1985-2014). The data were then categorized based on disease categories where the efficacies of the herbal preparations were described. The number of research findings in each disease category was later determined and computed as a percentage of all research outputs for the period under review.

Results

Researchers over the years have been conducting studies on efficacy, safety, phytochemistry of Ethiopian medicinal plants. About 454 published articles on Ethiopian medicinal plants in peer reviewed journals were recorded among which 75.4% were on biological activities. The trend in publication shows increment in the recent years. Figure 1 shows increasing trend in the number of published articles on Ethiopian traditional medicinal plants in Ethiopia. Trend of publication by Ethiopian Public Health Institute (EPHI) is also presented.



Note: EPHI- Ethiopian Public Health Institute

Figure 1: Number of Publications of Ethiopian medicinal plants in peer reviewed journal (1985-2014)

As shown in Table 1, the most research activities done in medicinal plants within the specified period were on anti-microbial activities (20.8%), antiprotozoal activity (13.7%) and ethnomedicinal study (9.76%). Other areas where research activities moderately undertaken were on formulation (3.1%), antidiabetic (2%), larvicidal (2%), mental illness (2%) and antimycobacterial (1.8%).

The most researched medicinal plants were *Moringa stenopetala, Glinus lotoides, Lippiaa doensis* and *Phytolacca dodecandra* for their pharmacological activity (efficacy and safety) and phytochemical analysis (Table 3). Parts of the medicinal plants used in the research were leaves (42.0%) followed by roots (15.4%) (Table 2).

Table 1: Categorization of scientific research on Ethiopia traditional medicine from 1985-2014

Se No.	Category	Number of publications	%
1	Knowledge, Attitude and Practice	42	7.6
2	Phytochemistry	36	6.5
3	Formulation (solid, semi-solid and liquid)	17	3.1
4	Ethnomedicinal study of medicinal plants (Human and Veterinary)	54	9.7
5	Toxicity (chronic/sub chronic, dermal)	19	3.4
6	*For agents causing Communicable diseases (Human)*		
6.1	Antimicrobial activity (Antibacterial, Antiviral, antifungal activity*)*	115	20.8
6.2	Antiprotozoal activity	76	13.7
6.3	Antimycobacterial activity	10	1.8
6.4	Wound healing activity	5	0.9
7	*Non-communicable diseases (Human)*		
7.1	Mental illness /anxiolytic	11	2.0
7.2	Ant diabetic activity	11	2.0
7.3	Diuretic effect	6	1.1
7.4	Anti-asthmatic effect	6	1.1
7.5	Antihypertensive activity	5	0.9
7.6	Anticancer activity	2	0.4
8	*Insecticidal/repellant activity*		
8.1	Repellent activity	10	1.8
8.2	Insecticidal activity	17	3.1
8.3	Larvicidal activity	11	2.0
9	*Others*		
9.1	Anthelmintic activity	18	3.2
9.2	Anti-inflammatory, analgesic and antipyretic activity	42	7.6
9.3	Antioxidant and Hepatoprotective	25	4.5
9.4	Gastrointestinal activity	10	1.8
9.5	Antifertility activity	5	0.9
9.6	Antirabies	1	0.2

Table 2: Plant parts used for research in Ethiopian traditional medicine from 1985-2014

Plant Parts	Number	%
Leaf	87	41.8
Root	37	17.8
Aerial parts	17	8.2
Seed	15	7.2
Steam bark	14	6.7
Fruits	8	3.8
Flowers	2	1.0
Essential oil	16	7.7
Hydrosol	6	2.9
Latex	2	1.0
Others (Oleoresin, Berry, Scented myrrh etc)	4	2.0

Table 3: Medicinal plants most researched on Ethiopia traditional medicine from 1985-2014

Number	Medicinal Plant species	Number of publications	%
1	*Moringa stenopetala*	15	4.2
2	*Glinus lotoides*	8	2.2
3	*Lippia adoensis*	8	2.2
4	*Phytolacca dodecandra*	7	1.9
5	*Albizia gummifera*	6	1.7
6	*Artemisia afra*	6	1.7
7	*Croton macrostachyus*	6	1.7
8	*Rumex abyssinicus*	6	1.7
9	*Ajugaremota*	5	1.4
10	*Artemisia abyssinica*	5	1.4
11	*Bersama abyssinica*	5	1.4
12	*Calpurnia aurea*	5	1.4
13	*Milletia ferruginea*	5	1.4
14	*Ocimum basilicum*	5	1.4
15	*Ocimum lamiifolium*	5	1.4
16	*Ocimum suave*	5	1.4
17	*Plumbago zeylanica*	5	1.4
18	*Thymus schimperi*	5	1.4
19	*Artemisia rehan*	4	1.1
20	*Asparagus africanus*	4	1.1
21	*Clerodendrum myricoides*	4	1.1
22	*Cymbopogon citratus*	4	1.1
23	*Echinops kebericho*	4	1.1
24	*Foeniculum vulgare*	4	1.1
25	*Syzigum guineense*	4	1.1
26	*Verbascum sinaiticum*	4	1.1
27	*Warburgia ugandensis*	4	1.1

Discussion

The pattern of research output is skewed towards anti-microbial, antiprotozoal, ethnomedicinal and phytochemistry, while medicinal plants for non-communicable diseases such as hypertension diabetics, cancer and mental illness are still growing. This may be due to many reasons. The first could be the magnitude of the problems in the country. For example malaria is a common public health problem in Ethiopia affecting about 68% of the population (FMoH 2011), Ethiopia is also one of the 22 high TB burden countries in the world (Kebede et al. 2014; WHO 2011) and non-communicable diseases are also emerging in Ethiopia accounts for 30 percent of total deaths (WHO 2014).

The second reason may be due to the emergence of antimicrobial and antimalarial drug resistances. Antimicrobial drug resistance has increasingly been recognized as a growing global health threat (WHO 2012). The resistance encompasses a range of pathogens including bacteria, mycobacteria, viruses, fungi and parasites (Gebre-Yohannes and Limenih 1980; Roma et al. 2000; Drug Administration and Control Authority of Ethiopia 2009; Falagas and Karveli 2006; Lamboro et al. 2016). This may also have triggered a plethora of research interests in such areas with the hope of finding new and improved method of treatment. The third reason may be due to inadequate facilities, funds, and limited technical know-how to conduct researches in threats like tuberculosis, cancer and HIV/AIDS.

The second most important research area in Ethiopian traditional medicine as per this review was ethnobotanical documentation. Conveyance of the knowledge on traditional medicine from person to person based on cultural rules of age, gender, lineage or religious disciple leads, to the fast disappearance of ethnophamacological information even though mostly tacit in nature (Abebe 1996; Nolan 1998). In view of the rapid loss of the knowledge, its documentation as well as better understanding of its botanico-historical roots has become an essential task of ethno-allied disciplines (Leonti et al. 2003; Hue et al. 1998). This review showed that Ethiopia is in a good start for documenting the indigenous knowledge and of sustainable medicinal plant biodiversity conservation even if a lot more is expected. Isolation and characterization of natural products yielded several compounds for the development of new drugs where research has not been adequately carried out to maintain the health of human beings (Odukoya 1998; Sasidharan et al. 2011). Research activities in similar fields are relatively meager in Ethiopia.

Several studies showed that leaves are the predominant plant parts that healers and the community commonly utilize (Giday et al. 2009; Osemene et al. 2011; Tekle 2014).Utilization of leaves and seeds has less negative impact on sustainable utilization of the plants.This is in line with a study conducted in different African countries (Mosihuzzaman and Choudhary 2008; Thillaivanan and Samraj 2014).

Conclusion and recommendations

Understanding of the trend in traditional medicinal plant use is critical for the design of directions for research and development. There is need for rapid enhancement of research, development and commercialization of Ethiopian herbal based medicinal products. Government support can play a vital role in establishment of traditional medicine council, execution of policies and workable strategies which involves the traditional healers, universities, research institutions local manufacturing industries and other stakeholders. There is need for further study especially in important areas that have recorded less research outputs.

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Unintended pregnancy and associated factors among Ethiopian women

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Abstract
Background: Unintended pregnancy is a pregnancy that is either mistimed or unwanted at the time of conception. From 210 million pregnancies that occur each year globally, 80 million are unintended which 42 million results in induced abortions.
Objective: To determine the magnitude and identify associated factors of unintended pregnancy among 15-49 years Ethiopian women who had a live birth within five years preceding the survey.
Method: Ethiopia Demographic and Health Survey 2011 data was used**.** A total of 7759 women were included in the analysis. Association between unintended pregnancy and the categorical independent variables was assessed using Bivariate and Multivariable logistic regression analysis at 95% confidence level by STATA ver.11.0 statistical software.
Result: Approximately one fourth of the women had unintended pregnancy. Women in the age group of 15-24 (AOR=2.18; 95%CI: 1.737, 2.732), never in union (AOR=6.85; 95%CI: (3.758, 12.507), having more than 4 children AOR=2.25; 95%CI: 1.832, 2.769) and who know modern contraception method (AOR=2.37; 95%CI: 1.588, 3.525) were more likely to have had unintended pregnancy.
Conclusion: Unintended pregnancy is still a public health problem in Ethiopia that can lead to unsafe abortion and higher child and maternal mortality. Education should be given on outcomes of unintended pregnancy and on the availability, access and use of family planning.

Introduction
Unintended pregnancy is a pregnancy that is either mistimed or unwanted at the time of conception (Amyo et al. 2010; Santelli et al. 2007). It remains a common public health problem worldwide. Globally, about 80 million unintended pregnancies are estimated from 210 million pregnancies which occur each year; 22 million of these are unsafe abortion and 18.5 million of these cases occur in developing countries (WHO 2008; WHO 2011; UNDP 2012).

Approximately 14 million unintended pregnancies occur every year in sub-Saharan Africa (Hubacher et al. 2008). In Ethiopia, an estimated 42% of all pregnancies were unintended. The outcome of this unintended pregnancy was 14% mistimed birth, 12% unwanted birth, 10% ending in induced abortion (Guttmacher 2010). According to Ethiopian Demographic Health Survey (EDHS) 2005, thirty five percent of the pregnancies were unintended (CSA 2006). Unintended pregnancy is the root cause of abortion and also causes to numerous maternal and child health problems like low birth weight, infant mortality, childhood mental and physically health problems. Not using contraception, inconsistent or incorrect use of contraception, contraceptive failure, nonconsensual sex, socio-economic and socio-demographic factors are reported to affect the prevalence of unintended pregnancy (Guttmacher 2013; Hoodraych 2012; Maria 2013; Seged et al. 2007). Even though facility and community based studies related to unintended pregnancy were done in in different areas of the country, contributing factors for this were not identified well. Therefore, this study was aimed to identify the major factors associated with unintended pregnancy in Ethiopia.

Materials and Methods
The data for the study was obtained from the third EDHS 2011 which was conducted by (CSA). It was a cross-sectional and nationwide sample survey which was designed to collect information by interviewing reproductive age women regarding to their fertility desire and family planning. Stratified sampling procedure was achieved by separating the urban and rural areas of each region. A total of 624 EAs, 187 urban and 437 rural areas were designated and from each EA 30 households were selected (www.infoplease.com/country /Ethiopia). Household, women's, and men's questionnaires were used to collect information and individual EDHS data set record found in the MEASURE DHS website was used. Variables with greater than 5% missing values/not applicable were excluded; categorization and re-categorization for continuous and categorical variables were applied. Socio-demographic characteristics were described in descriptive statistics. Bivariate and Multivariable logistic regression was used to show the magnitude and direction of association.

P-value less than 0.05 were considered for statistical significance. Variance Inflation Factor (VIF) with value less than 10 considered as no Multicollinearity between variables (Hosmer and Lemeshow 2000). Then model goodness of fit and model prediction power was checked by Hosmor-Lemeshow statistic and Receiver Operating Characteristics (ROC) Curve respectively. Confounders and interaction effect was checked by deviance statistics using likelihood ratio test. Finally the model specification was checked by link test. STATA ver.11statistical software was used for all kinds of analysis. Ethical clearance and permission to use the EDHS 2011data were obtained from the Ethical Review Committees of Mekelle University and Measure DHS project online respectively.

Results

From a total 7759 women's who had a live birth, 1999 (25.76%) of them had unintended pregnancy, 6247(80.51%) were live in rural areas, 7041 (90.75%) were currently in union, 3796(48.92%) were found within the age group between 25-35years, 3358(43.30%) were Muslim and 3187(41.08%) women had 0-2 children were (Table1). The highest percentage of women was none educated 5180 (66.76%) and not employed 5306 (68.47%). Majority of women 7307(94.17%) knew modern contraceptive method and above half 4625 (59.61%) of the women have never used any contraceptive method. Women who had big problem of reaching the health facility were 5559(71.71%). More than half of women 4288(55.26%) had no ANC visit during their pregnancy (Table 2).

Associated factors of unintended pregnancy: The factors associated with unintended pregnancy were number of living children, age, employment, marital status, and women educational level, ever used any contraceptive method, knowledge of any contraceptive method, and unmet need.

Table 2. Socio-demographic characteristics of women aged 15-49, EDHS 2011

Variables	Categories	Frequency	Percentage
Place of residence	Urban	1512	19.49
	Rural	6247	80.51
Region	Tigray	847	10.92
	Afar	713	9.19
	Amhara	965	12.44
	Oromia	1100	14.18
	Somalia	559	7.20
	Benshangul-Gumuz	673	8.67
	SNNP	1052	13.56
	Gambela	607	7.82
	Harari	440	5.67
	Dire-Dawa	456	5.88
	Addis-Ababa	347	4.47
Women age	15-24	2012	25.93
	25-34	3796	48.92
	35-49	1951	25.15
Husband age	<=30	2396	34.16
	31-40	2741	39.07
	>=41	1878	26.77
Number of living children	0-2	3187	41.08
	3-4	2306	29.72
	>4	2266	29.20
Religion	Muslim	3358	43.30
	Orthodox	2693	34.73
	Protestant	1478	19.06
	Others	226	2.91
Marital status	Currently In union	7041	90.75
	Formerly in union	652	8.40
	Never in union	66	0.85
Husband desire for children	Both wants the same	2967	42.30
	Wants different	2254	32.13
	Don't know	1794	25.57

Table 3: Socio-economic characteristics and health related information of women aged 15-49, EDHS 2011

Variables	Categories	Frequency	Percentage
Wealth index	Poor	3631	46.80
	Middle	1239	15.97
	Rich	2889	37.23
Currently employed	No	5306	68.47
	Yes	2443	31.53
Women education	No education	5180	66.76
	Primary	2094	26.99
	Sec/higher	485	6.25
Unmet need	No	3150	40.65
	Yes	2010	25.94
	Using contraceptive	1892	24.42
	Others	697	8.99
Knowledge of any contraceptive method	Knows no method	420	5.41
	Knows only traditional	32	0.42
	Knows modern	7307	94.17
Ever used any contraceptive method	No	4625	59.61
	Yes	3134	40.39
Exposure to Radio/TV	No	5935	76.49
	Yes	1824	23.51
Distance to health facility	Big problem	5559	71.71
	Not big problem	2193	28.29
ANC visit	No	4288	55.26
	Yes	3471	44.74

Women in the age group of 15-24 were 2.18 times more likely (AOR=2.18, 95%CI; 1.737, 2.732) to have unintended pregnancy compared to those found in the age group of 35-49. Having more than 4 children was 2.25 times (AOR=2.25, 95%CI; 1.832, 2.769) more likely to have had unintended pregnancy compared to having 0-2 children. Women's who were never in union were 6.85 times (AOR=6.85, 95%CI; 3.758, 12.507) more likely to have had unintended pregnancy than those currently in union. Uneducated women 39% (AOR=1.39, 95%CI; 1.073, 1.824) and primary 39% (AOR=1.39, 95%CI; 1.071, 1.797) were more likely to have had unintended pregnancy compared to those have secondary/higher education.

Regarding to the contraceptives use, those who ever used any contraceptive method were 35% more likely to have had unintended pregnancy compared to never used any contraceptive method (AOR=1.35, 95%CI; 1.132, 1.620). Women's who had knowledge of modern contraceptive were 2.37 times more likely to have had unintended pregnancy than those who knew no method (AOR=2.37, 95%CI; 1.588, 3.525). Women who had met need were 97% (AOR=0.03, 95%CI; 0.028, 0.044) less likely to had unintended pregnancy compared to those had unmet need.

Final fitting model information

Model prediction power: The prediction power of the model was (ROC=82.41%), which indicate model with these variables have very high power to predict unintended pregnancy.

Model Goodness of fit: The Hosmer-Lemeshow test shows insignificant (p-value=0.1524)

Multicollinearity: Multi-collinearity test statistics result shows no multi-collinearity (VIF=1.33).

Model specification: link test result of -hat square is non significant (P-value=0.117)

Confounder and interaction: Age and number of living children was confounded to each other and age was included in the final model. There were found no interacted variables.

Table 3: Factors associated with unintended pregnancy in 15-49 years age women, EDHS 2011

Variables		COR	95%CI	AOR	95%CI
Place of residence	Rural	1			
	Urban	1.18	1.045,1.343**	-	-
Region	Addis-Ababa	1		1	
	Tigray	0.51	0.384,0.677***	0.59	0.426,0.817**
Pv<0.001	Afar	0.17	0.119,0.244***	0.39	0.260,0.592***
	Amhara	1.09	0.838,1.416	1.17	0.864,1.599
	Oromia	1.28	0.989,1.651	1.33	0.987,1.793
	Somalia	0.15	0.104,0.231***	0.23	0.150,0.369***
	Benshangul-Gumuz	0.80	0.603,1.060	0.97	0.695,1.350
	SNNP	0.78	0.601,1.018	0.84	0.619,1.146
	Gambela	0.70	0.521,0.933*	1.03	0.737,1.448
	Harari	1.10	0.815,1.483	1.10	0.786,1.536
	Dire-Dawa	0.81	0.598,1.101	0.89	0.634,1.260
Number of living	0-2	1		1	
children	3-4	1.03	0.913,1.173	1.51	1.271,1.798***
Pv<0.001	>4	1.37	1.211,1.544***	2.25	1.832,2.769***
Age	35-49	1		1	
Pv=0.893	15-24	0.99	0.859,1.136	2.18	1.737,2.732***
	25-34	0.84	0.748,0.959**	1.24	1.057,1.474**
Religion	Muslim	1			
	Orthodox	1.70	1.510,1.905***	-	-
	Protestant	1.32	1.145,1.523***	-	-
	Others	1.41	1.042,1.913*	-	-
	Currently in union	1		1	
Marital status	Formerly in union	1.52	1.284,1.811***	1.39	1.025,1.897*
	Never in union	7.56	4.428, 12.924***	6.85	3.758,12.507***
Women education	Sec/higher	1		1	
Pv<0.001	No education	0.99	0.801,1.239	1.39	1.073,1.824*
	Primary	1.42	1.132,1.787**	1.39	1.071,1.797*
Husband desire for	Both wants same	1			
children	Wants different	0.98	0.866, 1.114	-	-
Pv=0.012	Don't know	0.82	0.720,0.950**	-	-
Wealth index	Poor	1		1	
	Middle	1.58	1.362,1.826***	1.05	0.883,1.252
	Rich	1.68	1.505,1.886***	1.19	1.024,1.390*
Currently employed	No	1		1	
	Yes	1.55	1.394,1.725***	1.22	1.074,1.384**
Knowledge of any	Knows no method	1			
contraceptive	Knows traditional	1.07	0.311, 3.684	1.51	0.335,6.824
Pv<0.001	Knows modern	3.79	2.695, 5.334***	2.37	1.588,3.525***
Ever used any	No	1		1	
contraceptive	Yes	1.92	1.734,2.133***	1.35	1.132,1.620***
Unmet need	Yes	1		1	
	Using contraceptive	0.55	0.481,0.623***	0.38	0.315,0.461***
	No	0.03	0.025.0.039***	0.03	0.028,0.044***
	Others	0.50	0.412,0.592***	0.37	0.267,0.505***
Distance to health	Big problem	1			
facility	Not a big problem	1.14	1.028,1.284*	-	-

*COR- crude odds ratio, AOR-adjusted odds ratio, CI-confidence interval, 1-reference, *P-value<0.05, ** P-value<0.01, *** P-value<0.001, Pv-the overall significance- a variable with different significance categories, overall significance was calculated.*

Discussion

In this study, 25.76% of Ethiopian women had unintended pregnancy which is similar with studies done in Kenya (24%) and Eastern Ethiopia (27.9%) (Lawrence et al. 2013; Kassa et al. 2012). Study in Harari (33.3%) and Jimma (39%) showed higher prevalence than this study (Solomon and Mesganaw 2006; Dibaba 2010). This could be underestimated because the respondent may change their intention to their previous pregnancy after they have given birth

or may be not interested to report having unintended pregnancy in the current study. Number of living children, marital status, age and education were significantly associated factors which contribute to had unintended pregnancy according to the current study. The more children the women had; the more likely to have unintended pregnancy. This is similar with findings in Ecuador and Jimma (Migual and Isabel 2010; Dibaba 2010). Never-in- union women were more likely to have unintended pregnancy which is in line with studies from New Zealand, Ecuador and Harari Ethiopia that showed being single women were more likely than married to have un intended pregnancy (Amin et al. 2009, Migual and Isabel 2011; and Solomon and Mesganaw 2006). Moreover, similar finding was found in Kenya, in which currently married and formerly married were less likely to experience unintended pregnancy compared to never-in- union (Lawrwnce et al. 2013). Women within 15-24 years age group were report their pregnancy as unintended which was consistent with findings in Kenya and Harari in which teenagers were more likely to had unintended pregnancy (Lawrwnce *et al*. 2013; Solomon and Mesganaw 2006).

Women with no education and primary education level were more likely to have unintended pregnancy which is similar with the studies done in Ecuador (Migual and Isabel 2011) and in Harari Ethiopia, in which unintended pregnancy decreases as education level increases (Solomon and Mesganaw 2006) but a study conducted in Bangladesh showed the insignificant effect of education for unintended pregnancy (Kemal and Islam 2011).

Women who had knowledge of modern contraceptive, unmet need and ever used any contraceptive method were also revealed as factors for unintended pregnancy. This finding is supported by Bangladesh in which women who ever used modern methods were more likely to have had unintended pregnancy (Kemal and Islam 2011) but opposite to a study conducted in Nepal which showed that women who had higher level of knowledge of FP method were less likely to have had unintended pregnancy (Adhikari et al. 2009). The difference could be due to use of different measurements to explain knowledge.

Conclusion and Recommendation

Approximately one fourth of the respondent had unintended pregnancy and this shows that unintended pregnancy is still a public health problem in Ethiopia that can lead to unsafe abortion and higher child and maternal mortality. Having more than four children, never in union, being in the age group of 15-24 years, being uneducated, ever used any contraceptive method, having knowledge of modern contraceptive methods and unmet need for contraception were the most risk groups to have had unintended pregnancy. Incessant education should be provided on the use of family planning and outcomes of unintended pregnancy.

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Prevalence and distribution of *Salmonella* in slaughtered sheep at Addis Ababa municipal abattoir, Ethiopia

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Abstract
Introduction: *Salmonella* is one of the most significant enteric pathogen from food safety point of view in which it is classified among the major cause of food borne illnesses.
Objective: To determine the prevalence of *Salmonella* in slaughtered sheep and to identify underlying serogroups from slaughtered sheep at Addis Ababa municipal abattoir, Ethiopia.
Methods: A cross-sectional study was conducted to identify and isolate *Salmonella* serogroups following the techniques recommended by the International Organization for Standardization 6579. Liver, abomasums and mesenteric lymph node tissue samples were collected aseptically using sterile scalpel blade in a sterile plastic bag. Prevalence was calculated and serotyping was done using rapid slide agglutination test.
Results: Of 50 animals examined, one or more of the samples were *Salmonella* positive in 8 (5.33%) of the animals and from a total of tissue samples examined, 9 (6%) were Salmonella positive. Of the nine *Salmonella* isolates, three different serogroups were identified of which serogroup B (O4) was predominant (77.8%) followed by Serogroup C2 (O8) (11.1%) and Serogroup D (O9) (11.1%).
Conclusion: *Salmonella* was widespread in tissue samples of slaughtered sheep carcasses in the Addis Ababa abattoir. Sources of pathogens in food animals need to be investigated and further study of *Salmonella* and other enteric pathogens in the public food chain is recommended. Also their food safety implication has to be further evaluated.
Keywords: Prevalence; *Salmonella*; sheep carcasses

Introduction
Salmonellosis is an infectious disease of humans and animals caused by organisms of the two species i.e. *Salmonella enterica* and *S. bongori. Salmonella enterica*, a Gram-negative, non-sporing, catalase-positive, oxidase-negative facultative anaerobic bacillus is a significant cause of morbidity and mortality in humans and animals, with multidrug-resistant *S. enterica* serovar *typhimurium* being an emerging problem (Gabert et al. 1999). Numerous *Salmonella* serovars cause acute and chronic enteritis, acute septicemia or subclinical infections in animals and humans. *Salmonella* species have also been extensively incriminated worldwide as common causes of bacterial gastroenteritis in humans, with food-animals serving as important reservoirs (Acha and Szyfres 2001). The epidemiology of the disease is complex and expected to vary with change in the pathogens themselves, industrialization, urbanization and change of lifestyles, knowledge, belief and practices of food handlers and consumers, demographic changes, international travel and migration, international trade in food, animal feed and in animals and poverty and lack of safe food preparation facilities (Altekruse et al. 1998).

Food-borne illnesses, including salmonellosis, are widespread and have an impact on communities in both the developing and developed world. In industrialized countries the incidence of salmonellosis is on the rise due to the emergence and increase of *S. enteritidis* and *S. typhimurium* DT 104 (Wray and Davies 2000). Interest in *Salmonella* has heightened in recent years due to the increased susceptibility of AIDS patients to Salmonellosis, the devastating effects of *S. enteritidis* in the poultry industry, and the globalization of agricultural trade. Persistent and severe salmonellosis has also been recognized as a problem among patients with AIDS (Clarke and Gyles 1993). Recent studies (DiMarzio et al. 2013) also showed that in the developed countries, the number of antibiotic-resistant isolates identified in humans is steadily increasing, suggesting that the spread of antibiotic resistant strains is a major threat to public health. Contaminated food of animal origin, particularly meat products are also an important source of *S. typhimurium* in human infections. *S. typhimurium* has been described as a collection of variants that vary significantly in their host range and their degree of host adaptation. It is the third most common serovar causing human food-poisoning in some parts of the world (Alemayehu et al. 2002). The disease can affect all species of domestic animals; young animals and pregnant animals are the most susceptible. Many

animals especially, poultry and pigs could be infected but show no clinical illness (Wray and Davies 2000). Human carriers of the pathogen are of concern to the food manufacturing and food service industries because of the perceived risk of contamination of food by infected food handlers and the risk of food-borne disease outbreaks (Mache et al. 1997). The presence of even small numbers of Salmonella in carcass meat and edible offal may lead to heavy contamination of the already processed and packed meat for distribution. When meat is cut into pieces more microorganisms are added to the surfaces of exposed tissue. Raw meats particularly minced meats have very high total counts of microorganisms and *Salmonella* are likely to be present in large numbers. Hence, monitoring the prevalence of *Salmonella* at abattoirs is imperative for creating a data bank and for effective control of such pathogens before they further enter in to the food chain (Molla and Mesfin 2003).

Materials and Methods

Study design: A cross sectional study design was used for the determination of Salmonella prevalence in organ samples derived from slaughtered sheep at Addis Ababa municipal abattoir from October 2013 to March, 2014.

Sample Collection: Samples were collected aseptically using sterile scalpel blade to cut the desired amount of tissue samples (25g) and sterile plastic bag was used to hold the samples from slaughtered sheep carcasses during slaughtering operations. Sample was collected from slaughtered animals showing characteristic lesions like enlarged fatty and congested liver, inflamed abomasum and enlarged, moist or bleeding mesenteric lymph nodes (three types of samples from each carcass: liver, abomasum and mesenteric lymph nodes) were collected once in a week and a total of 150 samples were collected from 50 sheep carcasses slaughtered during the study period. The connective tissue and fat were trimmed from the mesenteric lymph nodes before mixing and pre-enrichment. Samples in sterile containers were put in an icebox and transported immediately to the food microbiology laboratory of the EPHI, Addis Ababa, for further processing and laboratory analysis.

Isolation and identification of Salmonella

Culture Methods: Isolation and identification of S*almonella* was performed following the techniques recommended by the International Organization for Standardization 6579 (ISO 1998; Quinn et al. 1994). Twenty-five gram of each sample was weighed and organ samples were cut into smaller fine pieces using sterile scalpel blades. The prepared sample was put in a stomacher bag and to each of the 25 gm sample; 225 ml of buffered peptone water (BPW) (Park, Northampton, UK) was added. An amount of 9ml of BPW for each gram of sample (1:9 sample weights to BPW volume ratio) was used for pre enrichment of samples, which were below 25 gm. Each sample in the stomacher bag with the pre-enrichment medium (BPW) was macerated using a stomacher (Seward Stomacher 400, London) for 2 min at high speed and then incubated at 37 ^{0}C for 16-20 hrs.

A 0.1 ml of the pre-enrichment broth was transferred aseptically to 10 ml of Rappaport–Vassiliadis (RV) soya broth (Oxoid, England) and incubated for 18–24 hrs at 42 ^{0}C. A loop full of each enrichment broth was streaked onto xylose lysine deoxycholate agar (XLD) (Fluka, Spain) and incubated at 37 ^{0}C for 24 hrs. The plates were then examined for the presence of *Salmonella* colonies. Putative *Salmonella* colonies were sub cultured onto nutrient broth (Fluka, Switzerland) and subjected to different biochemical tests following standard methods of International Organization for Standardization 6579 (ISO 1998; Quinn et al. 1994).

Biochemical Tests: All suspected non-lactose fermenting *Salmonella* colonies were picked from the nutrient agar and inoculated into the following biochemical tubes for identification: triple sugar iron (TSI) agar, lysine iron agar, Simmon's citrate agar, urea agar and peptone water (indole) and incubated for 24 or 48 hours at 37°C. Colonies producing an alkaline slant with acid (yellow color) butt on TSI with hydrogen sulphide and gas production, positive for lysine (purple color), negative for urea hydrolysis (remained unchanged), negative for tryptophan utilization (indole test) (yellow-brown ring), and positive for citrate utilization (blue color) were considered to be *Salmonella*-positive (International Organization for Standardization 6579 (ISO 1998; Quinn et al. 1994).

Serology: Positive colonies of putative Salmonella organisms were further tested for agglutination by rapid slide agglutination test using Salmonella polyvalent antisera set 1 (Poly O, Poly O1 and VI) (Mast diagnostics, Mast group Ltd, Merseyside, UK). Suspected colonies grown on nutrient agar were mixed with a drop of sterile saline solution and it was emulsified to produce a distinct uniform turbidity then a drop of polyvalent antiserum O was added and then reagents were mixed by tilting the slide back and forth for 60 seconds while viewing under indirect light against a dark back ground, it was considered as positive when agglutination was observed, and

negative if no agglutination. To exclude any spontaneous agglutination, a negative control using physiological saline solution and bacterial colony was done. *Salmonella* polyvalent antiserum O_1 was used when suspected bacteria fail to agglutinate with *Salmonella* polyvalent O antiserum I. Data was summarized using descriptive statistical methods.

Results

Prevalence and Distribution: Of the total 150 samples from 50 animals examined, *salmonella* was isolated in 9 (6%) samples of 8(16.0%) sheep carcasses. Based on culture methods and biochemical tests employed, *Salmonella* were isolated from abomasum and mesenteric lymph node samples with the highest proportion 6 (12%) from abomasum and 3 (6%) from the mesenteric lymph node. All liver samples were found Salmonella negative (Table 1).

Serotyping: Out of the total nine (9) Salmonella isolates, three (3) serogroups were identified. Serogroup B was the predominant (77.8%), followed by serogroup C2 and serogroup D (each 11.1%). The highest proportion (55.5%) of Serogroup B was identified from abomasum samples as compared to mesenteric lymph node (22.2%).

Table 1: Prevalence and Distribution of salmonella from sample sources

Sample type	Total no. examined	Positive no. (%)
Liver	50	-
Abomasum	50	6 (12)
Mesenteric lymph node	50	3 (6)
Total	150	9 (6)

The distribution of serogroups in the various tissue samples showed that serogroup B was the most prevalent in which out of nine positive *Salmonella* samples, it was detected in seven (5 abomasum and 2 mesenteric lymph node) samples. *Salmonella* serogroup C_2 (11.0%) and D (11.0%) were detected only from one mesenteric lymph node and one abomasum samples respectively (Table 2).

Table 2: Distribution of Salmonella serogroups isolated from slaughtered sheep carcasses.

Salmonella Serogroup	Abomasum	Mesenteric lymph node	Total no. (%)
B (O4)	5	2	7 (77.8)
C_2 (O8)	-	1	1 (11.1)
D (O9)	1	-	1 (11.1)
Total	6	3	9 (100)

Discussion

The prevalence of *Salmonella* in slaughtered sheep in this study was found to be 16%. This is in line with studies done in central Ethiopia by Molla et al. (2006) and Wassie (2004) which was 11.5% and another study done in in Riyadh, Saudi Arabia (14.7%) (Nabbut and Al-Nakhli 1982) but relatively higher than a study done in Bishoftu abattoir by Woldemariam et al. (2005) which was 2.8%. The difference in the reported prevalence could be associated with the sampling procedures, sample type, distribution of *salmonella* in a lot examined and the method of detection employed (White et al. 2001). It is also known that keeping animals to be slaughtered in the abattoir's waiting pens crowded could facilitate the transmission of infection among the animals. Of the sample types taken from each animal during the study period, the mesenteric lymph nodes and abomasum samples proved to be useful indicators of infection, as most of the sheep and all *Salmonella* positive sheep were detected on the basis of those samples. The tissue prevalence distribution of *Salmonella* isolate was 6% in mesenteric lymph node which is in agreement with the finding of Molla et al. (2006) who reported 8.7% and 6.9% prevalence at Addis Ababa and Modjo abattoirs respectively. This finding is also supported by earlier observation by (Akafete and Haileleul 2011) who reported 5.6% prevalence at modjo export abattoir. However, the result is lower than the finding of Woldemariam et al. (2005) who reported 11.7% prevalence in mesenteric lymph node at Debre-Zeit ELFORA abattoir and slightly less than the finding of Wassie (2004) who indicated 7.7% at Addis Ababa and Mojo abattoirs. It is also consistent with the study conducted in apparently healthy slaughtered sheep in Australia (4%) in mesenteric lymph nodes (Moo et al. 1980). The findings of a high proportion of infected sheep (6%) harboring *Salmonella* in their mesenteric lymph nodes might be associated with the existence of high infection burden among Salmonella carrier animals and from animals acquiring new infection prior to slaughter due to different predisposing factors like starvation, overcrowding transportation and longer lairage confinement. The present high mesenteric lymph node prevalence of *Salmonella* also indicates

the existence of a substantial risk of cross-contamination during slaughtering, dressing and subsequent handling of the carcasses where fecal material contamination of the edible organs and carcass may occur during slaughtering operations unless hygienic measures are taken (Molla et al. 2006).

The prevalence of *Salmonella* in abomasum was found to be 12% which was significantly higher ($p<0.05$). Identification of higher proportion of *Salmonella* in abomasum might be due to fecal material contamination and poor hygienic status of working personnel, slaughterhouse and equipment during slaughtering operation. The present study also entails that liver samples did not appear to harbor *salmonella* on most occasions which is similar with studies conducted by Samuel et al. (1981), Nabbut and Al-Nakhli (1982) and Nyeleti et al. (2000). This indicates that the organisms did not spread beyond the lymph nodes or if they did, they were too small in numbers to be detected by the method used; the liver is usually free of *Salmonella* at slaughter, but surfaces can be contaminated during processing. The ultimate source of this contamination is likely to be the *Salmonella* present in the gastrointestinal tract and mesenteric lymph nodes of the same animal or other animals slaughtered on the same day (Samuel et al. 1981; Nabbut and Al-Nakhli 1982).

In the study *Salmonella* serogroup B (O4) (77.8%) was the most frequently isolated serogroup followed by Salmonella serogroup C2 (O8) (11.1%) and *Salmonella* serogroup D (O9) (11.1%) from sample sources. All of those serogroups were detected by Molla et al. (2003) from food animals, slaughter house personnel and retail meat products in Ethiopia. Serogroup B and C2 were also reported by Ejeta et al. (2004) in mutton from retail supermarkets in Addis Ababa, Ethiopia. Another similar study conducted by Molla et al. (2006) indicated that serogroup B and D were most prevalent in apparently healthy slaughtered sheep of central Ethiopia. Serogroup B was also reported by Woldemariam et al. (2005) in apparently healthy slaughterred sheep in Bishoftu. Serogroup B was the major serogroup among the present isolates in which it contains certain pathogenic serotypes like *S.typhimurium, S. enteritidis and S. Dublin* and others. The detection of all those serogroups which probably contains those pathogenic serotypes in apparently healthy slaughtered sheep is of paramount health significance since contaminated edible organs may pose serious public health risks.

Conclusion

The present study indicated that *Salmonella* was prevalent in sheep carcasses in Addis Ababa municipal abattoir. Different serogroups identified in this study reflects the possible cross-contamination from multiple sources at the slaughterhouse and poor hygiene during meat cutting and evisceration. *Salmonella* contamination was widespread in tissue samples of slaughtered sheep carcass at the abattoir and the magnitude of the problem is especially high in abomasum samples as compared to others which represent a real public health hazard. Thus, sources of pathogens in food animals need to be investigated and further study of pathogens in the food chain is also needed.

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In vivo insecticidal activity of formulations containing essential oils of *Cymbopogon citratus* and *Eucalyptus globulus* against *Melophagus ovinus* in sheep

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Abstract
Background: National insecticide dipping program has been performed in Ethiopia for the last decade on sheep and goat to control ectoparasites. However, there is still high burden of ectoparasite in the country. On the other hand, the widespread use of chemical insecticides has significant drawbacks including increased cost, handling hazards, threat to human health and environment and insecticide resistance. These facts prompted the search for new alternative insecticides from plants for the control of ectoparasite.
Objective: To determine insecticidal potency of essential oil formulations on sheep naturally infested by *Melophagus ovinus* (sheep ked).
Method: A randomized block design studies were used on naturally ked infested sheep (n=6) as treatment and control (negative/Tween 80 and positive/diazinon) groups to evaluate insecticidal activity of two essential oils from *Cymbopogan citratus* and *Eucalyptus globulus* against *Melophagus ovinus* ectoparasite.
Result: The essential oils showed promising insecticidal activity against sheep ked. *C. citratus* essential oil has higher antiparasitic activity than *E. globulus* essential oils. *C. citratus* oil showed 94.7%, 87%, and 90.2% parasite reduction of sheep ked parasite at a concentration of 0.6254%, 0.3125% and 0.1563%, respectively. The *C. citratus* essential oil has no differential (p>0.05) insecticidal activity compared to diazinon at a concentration of 0.6254% and 0.1563%. Moderate antiparasitic activity was recorded in the groups of sheep treated by *E. globulus* essential oil.
Conclusion: Essential oils from *C. citratus* and *E. globulus* are potential plant sources of insecticides that can substitute diazinon for the management of *M. ovinus* in sheep. It is recommended that essential oils from *C. citratus* and *E. globulus* can be potential bio-rational insecticides in eco-friendly control of ectoparasite in sheep.

Key Words: *Cymbopogan citratus*, essential oil, *Eucalyptus globulus*, insecticide, *Melophagus ovinus*

Introduction
Ethiopia has the largest livestock population in Africa with 54.3 million cattle, 25.5 million sheep and 22.78 million goats (CSA 2011). As a result the country has huge leather production potential with 2.4 million hides, 10 million sheepskins and 7.4 million goat skins annually (MoA and ILRI 2013). The sector has been the backbone of the country's economy by contributing large share to the GDP of the country through the export of finished and semi-finished products to the world, beside, supplying 16-18 million skins and hides for local tanneries per annum (MoA and ILRI 2013). However, today, the benefits that this sector provide for the country is deteriorating mainly because of the rejection and down grading due to low quality raw materials (Kebede and Fetene 2012), mainly due to antemortem and postmortem factors, including poor animal husbandry, disease and parasites, bad slaughtering and flaying and bad practicing during curing, collection, transportation, storage and general handling (Abadi 2000). Pre slaughter stage of production was responsible for 65% of these defects of which the majority was due to a disease caused by ectoparasite (Kebede and Fetene 2012; Teshome, 2016). Ectoparasitic skin diseases of domestic ruminants that are caused by lice, sheep ked, ticks and mange mites are among the major problems that result in serious economic loss to smallholder farmers, the tanning industries and the country at large (Teshome 2016). The prevalent ectoparasites on ruminants are tick (37.66%), mange (10.38%), lice (29.55%) and sheep ked (23.38%) (Teshome 2016). *Mellophagus ovinus* (sheep ked) which accounts for 32.57% infestation of sheep was the main ectoparasite causing defects followed by *Bovicola ovis* (22.28%) (Tadesse et al. 2011). Highly potent insecticides are routinely used in Ethiopia for the control of sheep ked infestation (Zewdie 2010). However, indiscriminate use of chemical insecticides has led to insecticide resistance in parasite populations (Wall 2007; Lifschitz et al. 2008; Kumar et al. 2011). Besides their continues application has led to a number of environmental and health problems (Wall 2007). Cognizant of high prevalence of sheep ked among sheep farmers and problems related to synthetic chemical insecticides; calls for search for alternative bio rational insecticides from

natural sources which are safe, effective and environmental friendly. The use of herbal medicines by traditional healers and the rural community at large for treatment of sheep ked parasite remains the main stay of health care system for the majority of the population in Ethiopia. A previous study reported promising insecticidal activity of *Cymbopogon citratus,* and *Eucalyptus globulus* essential oils against *M. ovinus (Gemeda* et al. 2014). However, the study was conducted *in vitro* and no data is available on the insecticidal activity of these essential oils against *M. ovinus* in sheep (*in vivo*). This study was therefore conducted to evaluate the insecticidal activity of *C.citratus* (DC.) Stapf (Poaceae), *Eucalyptus globulus* Labill (Myrtaceae) essential oils in naturally *M. ovinus* sheep ked infested sheep.

Material and Methods

Plant material collection, identification and extraction: *C. citratus* and *E. globulus* essential oils were extracted from aerial and leaf part, respectively in Wondo-Genet Agricultural Research Center, EIAR, Wendo Genet, Ethiopia. Fresh plant materials (250g) were placed in a 5L round-bottom distillation flask and the plant material was wetted with 3L distilled water. The essential oils were obtained by hydro-distillation using Clevenger-type apparatus continuously for 3 hours. The volatile oils were taken from the upper layer. The aqueous layer was further portioned using dichloromethane to extract and enrich the essential oil from the water layer. The organic layer (dichloromethane extract) was filtered and dried with anhydrous sodium sulfate and concentrated using rotary evaporator to give the crude essential oil.

Formulations: The formulations recipe of essential oils were optimized in the Formulation Laboratory of the Traditional and Modern Medicine Directorate, EPHI. The formulation recipes are as follows: Formulation 1: 2.5% *E. globulus* oil in 2% aqueous Tween 80; Formulation 2: 1.25% *E. globulus* oil in 2% aqueous Tween 80; Formulation 3: 0.625% *E. globulus* oil in 2% aqueous Tween 80; Formulation 4: 0.625% C. *citratus* oil in 2% aqueous Tween 80; Formulation 5: 0.3125% *C. citratus* oil in 2% aqueous Tween 80 and Formulation 6: 0.156% *C. citratus* oil in 2% aqueous Tween 80.

In Vivo Insecticidal Assay: Experimental study design recommended by World Association for the Advancement of Veterinary Parasitology was followed in this study (Holdsworth *et al.* 2006). Sheep of the same weight and age (6-12 months) with adequate natural infestation were bought from the Fiche Local market and housed indoors in individual cages and fed daily. Animals were acclimatized to the facilities and the feeding /regime for 14 days prior to the treatment. The animals were identified by uniquely numbered ear tag. A randomized block design was used to randomly allocate the animals (n=6) into treatment and control (negative/Tween 80 and positive/diazinon) groups. The groups were maintained in isolation for the duration of the study to avoid contact with animals in any other group. On day 0, the numbers of live sheep ked found on 40 hair partings (10 cm wide) on four body sites (neck, shoulder, withers, flank and rump) were counted and recorded for each animal. All the treatment groups were treated by the essential oils at day-0 and day-14. Positive controls were treated only once with Diazinon at the start of the experiment (day 0) at a dose rate of 0.001%. The groups were selected by lottery method for each treatment as follows: Group 1: 1.25% *E. globulus*; Group 2: Diazinon; Group 3: 0.625% *C. citratus*; Group 4: 0.15625% *C. citratus*; Group 5: 2% Tween 80; Group 6: 2.5% *E. globulus*; Group 7: 0.3125% *C. citratus*; and Group 8: 0.625% *E. globulus*. Sheep ked parasite counting was done every week for 56 days. At each time point the mean live sheep ked count was taken for each group and the results compared with that of the untreated control group and positive control group. Responses to the treatment were monitored on alternate days at the time of drug application in terms of mean parasite count, and percent parasite reduction. Personnel involved in the collection of efficacy data were masked to the treatment assignment of the animals.

Data analysis: The in vivo sheep ked counts (every week for 56 days after treatment application) were recorded. Repeated measure analysis of variance was used to test for the effects of time and the time-treatment interaction. One-way analysis of variance (using Minitab 16.0 software) was used to test for treatment effects on each day and treatment groups were compared to the controls using Tukey test with a family error rate of 0.05. A probability of $p<0.05$ was considered to be significant.

Results

Table 1 shows *M. ovinus* reduction effect of *C. citratus* oil at different concentration on sheep on various days' post treatment. As can be seen in Table1, differences between mean percent reductions as identified by Tukey's Tests are displayed. Different concentration of *C. citratus* essential oils has comparable effect of reducing *M. ovinus* parasite with Diazinon after 7, 14, 28 and 49 days post treatment of sheep. Compared to standard drug used in the study, *C. citratus* essential oil at a concentration of 0.3125% has statistically lower

($p<0.05$) toxic effects on *M. ovinus* parasite in sheep on 7, 21, 35, 42 and 56 days of post treatment. On the other hand, at all study points in day of post treatment, all essential oil concentrations have statistically no differential parasite reduction potency. The mean percentage reduction of parasite in sheep treated with *C. citratus* oils ranged between 73.8% – 94.7%, 38.2%-87.0%, 48.2%–90.2% at a concentration of 0.6254%, 0.3125% and 0.1563 respectively.

Table1: *In vivo* insecticidal activity of *C. citratus* essential oil formulation on naturally *M. ovinus* infested Sheep, Fiche, Oromia region, Ethiopia

		Treatment dose (v/v)				
	Day	2%T80	0.1000% D	0.6254% C	0.3125% C	0.1563% C
Sheep Ked percent reduction	7 (PT)	39.5±26.4^{c}	100.0±0.0^{a}	75.5±15.6^{ab}	38.2±21.2^{c}	48.2±24.6^{bc}
	14(PT)	36.1±21.9^{c}	100.0±0.0^{a}	73.8±14.9^{ab}	87.0±12.5^{ab}	61.6±22.1^{bc}
	21(PT)	25.2±20.3^{c}	100.0±0.0^{a}	91.5±9.0^{ab}	75.5±7.4^{b}	81.3±8.5^{ab}
	28(PT)	43.3±20.6^{c}	100.0±0.0^{a}	86.0±10.5^{ab}	72.9±13.6^{ab}	80.4±13.2^{b}
	35(PT)	36.1±16.8^{c}	100.0±0.0^{a}	93.2±5.5^{a}	61.5±6.8^{b}	84.4±10.1^{a}
	42(PT)	32.6±21.7^{c}	100.0±0.0^{a}	84.7±11.8^{ab}	66.5±10.6^{b}	65.6±23.2^{b}
	49(PT)	33.3±18.6^{c}	100.0±0.0^{a}	90.0±6.2^{ab}	70.9±15.9^{b}	86.6±13.7^{ab}
	56(PT)	38.0±16.3^{c}	100.0±0.0^{a}	94.7±4.6^{a}	71.9±7.7^{b}	90.2±11.9^{a}

C: C. citratus; D: Diazinon; PT: post treatment; T: Tween; Values are expressed as percent reduction mean ± SD; mean percent reductions that do not share letter in the same rows are significantly different (P<0.05)

As indicated in Table2, *E. globulus* essential oil has moderate insecticidal activity on *M. ovinus* on sheep. Analysis of parasite on sheep on 14th and 28th days after spray of different concentrations of *E. globulus* essential oil formulations in 2% Tween 80 on sheep have no differential ($p<0.05$) sheep ked reduction activity compared to Diazinon. The percent reduction of *M. ovinus* parasite in sheep at lowest concentration of *E. globulus* (0.625%) was statistically lower compared to Diazinon. The highest percentage of parasite reduction (82%) by this oil was observed at a concentration of 2.5% on 28th day post treatment of sheep, while the lowest was observed at 1.25% concentration of *E. globulus* oil on 7th day of post-treatment.

Table 2: *In vivo* insecticidal activity of *E. globulus* essential oil formulation on naturally *M. ovinus* infested sheep, Fiche, Oromia region, Ethiopia

		Treatment dose (v/v)				
	Day	2%T80	0.1000%D	2.5000%E	1.2500%E	0.6250%E
Sheep Ked percent reduction	7 (PT)	39.5±26.4^{bc}	100.0±0.0^{a}	43.9±7.6^{b}	9.5±12.3^{c}	49.8±26.3^{b}
	14(PT)	36.1±21.9^{c}	100.0±0.0^{a}	78.1±13.2^{ab}	38.9±29.3^{c}	53.9±29.7^{bc}
	21(PT)	25.2±20.3^{c}	100.0±0.0^{a}	62.5±17.8^{b}	61.5±17.0^{b}	54.2±21.9^{bc}
	28(PT)	43.3±20.6^{d}	100.0±0.0^{a}	82.3±14.5^{ab}	54.4±14.4^{bc}	43.2±20.6^{cd}
	35(PT)	36.1±16.8^{c}	100.0±0.0^{a}	68.6±14.0^{b}	48.4±25.3^{bc}	51.1±15.4^{bc}
	42(PT)	32.6±21.7^{c}	100.0±0.0^{a}	69.5±21.8^{b}	60.4±14.3^{b}	56.0±13.4^{c}
	49(PT)	33.3±18.6^{c}	100.0±0.0^{a}	71.2±17.5^{b}	67.7±9.3^{b}	54.0±18.4^{bc}
	56(PT)	38.0±16.3^{c}	100.0±0.0^{b}	78.9±16.6^{ab}	58.8±11.6^{bc}	38.0±16.3^{c}

E: E. globulus; D: Diazinon; PT: post treatment; T: Tween. Values are expressed as percent reduction mean ± SD; mean percent reductions that do not share letter in the same rows are significantly different (P<0.05)

The two aromatic plant essential oils tested in this study showed parasite reduction activity in sheep against *M. ovinus* (Fig 1). The figure demonstrates that *E. globulus* oil has considerable lower sheep ked reduction activity compared to *C. citratus*, though the concentration used for *E. globulus* (0.625%) is fourfold higher than that of *C. citratus* (0.1563%). A significantly high sheep ked parasite reduction was observed on day 35 and 49 by *C. citratus* essential oil compared to *E. globulus* oil. Analysis of the data revealed that *C. citratus* essential oil has comparable reduction effect to Diazinon while *E. globulus* essential oil showed significantly lower ($p<0.05$) percent reduction.

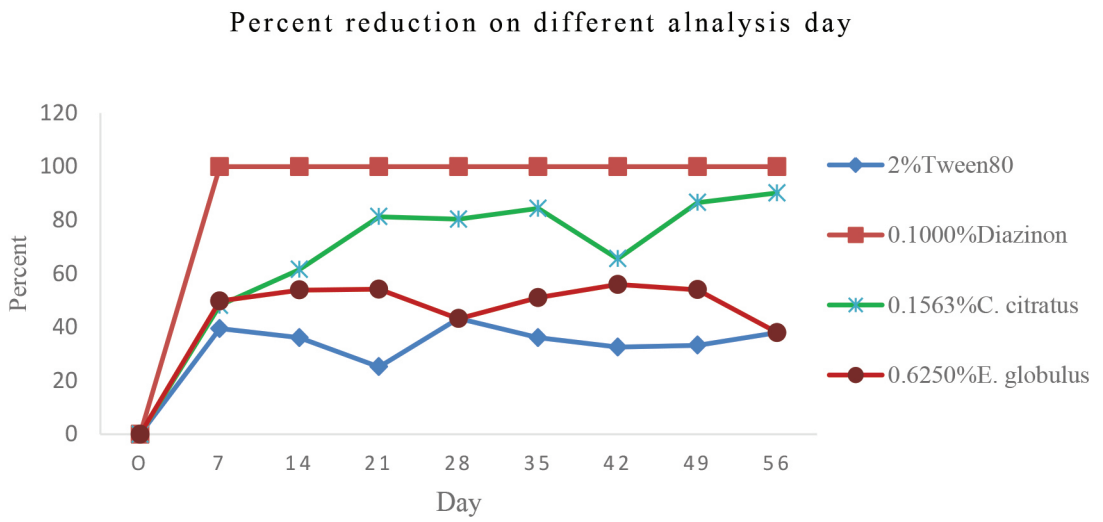

Figure 1: Percent reduction of *M. ovinus* parasite in sheep treated with *C.citratus*,*E.globulus* and Diazinon

Discussions

In the present study, six essential oil formulations from two medicinal plants were examined on sheep naturally infested by sheep ked. *C. citratus* essential oil has statistically comparable insecticidal activity in vivo with synthetic insecticide Diazinon. Synergy between the complex chemical constituents in the essential oil may be responsible for this insecticidal activity or may be due to potency of major compounds against *M. ovinus*. This finding showed *C. citratus* essential oil have similar activity with that of synthetic insecticide and can be the best candidate to alternate synthetic insecticides which corroborates the previous study in which showed *C. citratus* oil has comparable results with a synthetic insecticide Ivermectin (Gemeda et al. 2014). This finding also corroborates with the previous study of various researchers. Freitas et al. (2010), Vera et al. (2014) and Kabera et al (2011) reported insecticidal activity of *C. citratus* essential oils and its major constituents against adult and larvae of insects which could be attributed to its major constituents citral, or its isomers geranial and neral (Freitas et al. 2010). The essential oil and its constituents have recognized wide variety of biological activity against house fly (Sinthusiri and Soonwer 2013), tick (Singh et al. 2014), mite (Hanifah et al. 2011),pest (Ayvaz et al. 2010, Olorunnisola et al. 2014, Sonker et al. 2014), antimicrobial (Sonker et al. 2014), mosquito (Soonwera and Phasomkusolsi 2014) and leishmania (Machado et al. 2012).

Insecticidal activity was observed against *M. ovinus* sheep ked parasite by *E. globulus* essential oil in *in-vivo* model. Moderate parasite reduction by the essential oil from the eucalyptus may be due to phytochemicals including the major constituents 1, 8-cineole that could affect the nerve of the sheep ked leading to a disruption of vital system (Slimane et al. 2014). This finding was in line with previous study of Gemeda et al. (2014) that reported insecticidal activity of *E. globulus* oil against sheep ked. Song *et al*. (2016) also reported the effect of eucalyptus oils against stored mite and grain insects where they found fifty percent toxicity. The plant has insecticidal activity against egg, larvae and adult stage of insects (Maciel et al. 2009; Kumar et al. 2012). Moreover, other biological activities were reported including acaricidal activity (Pirali-Kheirabadi et al. 2009).

Superior insecticidal activity by *C. citratus* essential oils may be attributed to active compounds including mcyrecene, citronellal and neral found in the oils in addition to its major constituent citral. This oil has better insecticidal activity against sheep ked in the present study than *E. globulus* essential oil. The same conclusion has been reported in the study conducted by a previous study (Gemeda et al. 2014), where they reported high insecticidal activity of *C. citratus* essential oils against *M. ovinus*. In a similar study, Bossou et al. (2013), *C. citratus* has the best insecticidal activity against *Anopheles gambiae* compared with other tested essential oils.

Conclusion

This study that was conducted to evaluate *in vivo* insecticidal activity of two aromatic medicinal plants at three different concentrations against sheep ked showed promising results. The essential oil from *C. citratus* showed the highest insecticidal activity against *M. ovinus* at lowest concentration tested. Moreover, moderate insecticidal activity was

observed by *E. globulus* essential oil against *M. ovinus*. These aromatic plants could be potential sources of biorational insecticides against ectoparasites. Further study is needed to evaluate the stability of the formulations and dosage validation using community based study.

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