A multinomial modelling of the factors that impact viral load levels in adults on anti-retroviral therapy in Namibia: A case study of Nankudu Health District

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ABSTRACT

Namibia is one of the Sub-Saharan Africa countries still hit with the Human Immunodeficiency Virus (HIV), with a 12.6% HIV prevalence among adults aged 15-64 years, and with the Kavango West region of Namibia still having a 12.1% HIV infection prevalence. As a prevention in combating new HIV infections, viral load suppression is crucial, especially during the application of HIV treatment. In this paper, the multinomial logit regression model was used to statistically examine the impacting factors of Anti-Retroviral Therapy (ART) adult patients' viral load levels using data collected from the Nankudu health district. Results showed that majority of the patients had never been testing for Hepatitis B, regardless of their viral load levels, while patients' characteristics such as age, sex, marital status, ART duration, tuberculosis (TB) screening, hepatitis B screening, ART adherence and ART status had a significant impact on the patients' viral load levels. In addition, ART patients who were less than 20 years old and had been on ART between 4 to 15 years were more likely to have an undetectable viral load, while those who had been on ART between 7 to 15 years were less likely to have a virologic failure. Also, patients who were never tested for hepatitis B were more likely to have a virologic failure, while those who tested positive for TB were less likely to have an undetectable viral load. It is therefore recommended that the Namibian government and policy makers consider making hepatitis B screening mandatory, especially, among HIV patients and ART patients. Moreover, periodic viral load testing to monitor responses to ART is important and needs to be conducted to prevent progression of patients to virologic failure and drug resistance.

1 Introduction

As at the end of 2018, approximately 37.9 million people were living with the Human Immunodeficiency Virus (HIV) globally and Sub-Saharan Africa was home to more than half of this infected population (World Health Organization, 2020). According to the 2014 launched 90-90-90 goal of the United Nations' (UN) programme on HIV and Acquired Immuno-Deficiency Syndrome (HIV/AIDS), by 2020, 90% of all people living with HIV will know their HIV status, 90% of all people diagnosed with HIV infection will receive sustained Anti-Retroviral Therapy (ART) and 90% of all people receiving ART will have viral load suppression (Bain *et al.*, 2017). Achieving this targeted goal requires a viral load-informed care to ensure optimal HIV clinical follow-up and resistance monitoring (Bain *et al.*, 2017). AIDSinfo (2018) defines viral load as the amount of HIV in a sample of blood. To be precise, it is the number of HIV ribonucleic acid (RNA) copies per milliliter of blood.

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Achieving a viral load suppression is the ultimate goal of HIV treatment and the corner stone of strategies to achieve HIV epidemic control. Thus, viral load testing is crucial as it can be used to diagnose and confirm HIV treatment failure/success in a person. One of the goals of ART is to suppress a person's viral load to an undetectable level (a level too low for the virus to be detected by a viral load test) and such a person will be classified as faring well on the HIV treatment. The third 90 in the UNAIDS 90-90-90 goal requires that adults living with HIV on ART should reach an undetectable viral load of (less than) 40 copies of HIV per mL of blood. Having an undetectable (suppressed) viral load is associated with prolonged life and good health, and a person with such viral load cannot transmit the virus to another person. This viral load can be attained in 6 months after initiating treatment on the HIV person (Ali and Yirtaw, 2019).

Namibia is one of the Sub-Saharan Africa countries still hit with HIV, with a 12.6% HIV prevalence among adults aged 15-64 years in 2017 (ICAP, 2018). Even though several researchers and organizations such as ICAP (2018) have conducted studies to assess the impact of HIV in Namibia, these studies were mainly carried out before the country implemented the World Health Organization's treat-all strategy. This strategy recommends the HIV treatment of all people living with HIV. As such, information on the success of ART towards the 90-90-90 UNAIDS goal are still insufficient. Moreover, many studies have been carried out to identify contributing factors influencing HIV and its treatment in Namibia. However, factors contributing to viral load suppression, prevalence of low-levels viral load, clinical significance and management of low-levels viral load among ART patients still need to be sufficiently explored across regions in Namibia, especially in the Kavango West region with a 12.1% HIV infection prevalence rate. For this reason, the aim of this study was to examine the factors affecting the viral load levels of ART patients in the Nankudu health district in the Kavango West region. Findings from this study may provide feedback to site-level management such as clinicians and health workers who are responsible for quality HIV treatment and service deliveries in order to perhaps reduce new HIV infections.

2 Methodology

The data used in this paper were extracted from the Ministry of Health and Social Services' (MoHSS) directorate of special program electronic Patient Management System (ePMS) database from the 1st of January 2003 to the 31st of December 2018 for the Nankudu health district. This (secondary) database covers all the ART initiations at any health facility within the Nankudu health district from the 1st of January 2003 to the 31st of December 2018. Located in the Kavango West region of Namibia, this health district consists of twelve health facilities including a referral hospital and 4 health centres. A total of 6856 adult patients were initiated on ART between 1st of January 2003 to the 31st of December 2018 at this health district, as documented in the ePMS database.

For this study, the inclusion criteria were all patients aged 15-64 years who had been on ART for at least 6 months and had a viral load measurement, tuberculosis (TB) and hepatitis B screening results from the 1st of January 2003 to the 31st of December 2018 as documented in the ePMS database. Patients who were below the age of 15 years and above the age of 64 years, as well as patients who had been on ART for less than 6 months and had no viral load measurement, TB and hepatitis B screening results were excluded from this study. Ethical clearance was obtained from MoHSS directorate of special programs, prior to the commencement of this study. All revealing information about the patients' identities were already excluded from the data by MoHSS directorate of special programs before the data were collected by the co-author of this paper. All patients' records received from MoHSS directorate of special programs were anonymized to maintain the privacy of those whose records were part of this study.

2.1 Statistical Analysis

Regression analysis is a statistical techniques used to measure the effects of a set of predictor variables $\mathbf{X}(N \times P)$ on a response variable $\mathbf{y}(N \times 1)$. This effect measurement is done by solving the equation

$$\mathbf{y} = \beta_0 + \beta_1 \mathbf{x}_1 + \beta_2 \mathbf{x}_2 + \cdots + \beta_P \mathbf{x}_P + \mathbf{e}, \tag{1}$$

where $\mathbf{e}(N \times 1)$ is the error term, $\mathbf{x}_j(N \times 1)$, for $j = 1, 2, \cdots, P$, is the jth predictor variable, β_0 is the intercept term and β_j is the unknown coefficient term that needs to be estimated for the jth predictor variable. Equation (1) is a linear model (Oyedele, 2020) because it assumes that: (i) there is some linear function linking $\mathbf{y}$ to $\mathbf{X}$, (ii) both $\mathbf{X}$ and $\mathbf{y}$ are continuous variables and (iii) $\mathbf{y}$ follows a normal distribution with mean μ and constant variance σ^2. More often $\mathbf{y}$ is dichotomous in nature and non-normally distributed. In such situation, the modelling of $\mathbf{y}$ can be done using a non-linear model such as the generalized linear models. For the dichotomous $\mathbf{y}$, the natural distribution to consider would be the binomial distribution, i.e., $\mathbf{y} \sim \text{Bin}(n, p)$, where p is the probability of occurrence $(Y = 1)$ and n is the number of trials (Sparks, 2019).

A logit regression model can be used to estimate the probability of occurrence for the dichotomous $\mathbf{y}$ based on a set of predictor variables. In the logit model, the link function is obtained as

$$\text{logit}(Y = 1) = \log\left[\frac{P(Y = 1|\mathbf{X})}{1 - P(Y = 1|\mathbf{X})}\right]$$

and expressing $P(Y = 1|\mathbf{X})$ as a linear model (Equation (1)) yields

$$\log\left[\frac{P(Y = 1|\mathbf{X})}{1 - P(Y = 1|\mathbf{X})}\right] = \beta_0 + \beta_1 \mathbf{x}_1 + \beta_2 \mathbf{x}_2 + \cdots + \beta_P \mathbf{x}_P + \mathbf{e}, \tag{2}$$

making the logit model (Equation (2)) to have a more convenient odds ratio interpretation for its coefficient values (Sparks, 2019). However, if $\mathbf{y}$ has at least 3 (nominal) categories, then a multinomial logit regression model should be used to estimate the probability of occurrence (Milewska *et al.*, 2017). For $\mathbf{y}$ with G nominal categories, the multinomial logit model can be obtained as

$$\text{logit}(Y_g) = \log\left[\frac{P(Y = g|\mathbf{X})}{1 - P(Y = g'|\mathbf{X})}\right]$$

for $g = 1, 2, \cdots, G-1$, with g being the identified nominal category of $\mathbf{y}$ and g' the reference category. Expressing $P(Y = g|\mathbf{X})$ as a linear model yields

$$\log\left[\frac{P(Y = g|\mathbf{X})}{1 - P(Y = g'|\mathbf{X})}\right] = \beta_{g0} + \beta_{g1} \mathbf{x}_1 + \beta_{g2} \mathbf{x}_2 + \cdots + \beta_{gP} \mathbf{x}_P + \mathbf{e}_g, \tag{3}$$

where $g = 1, 2, \cdots, G-1$.

The predictor variables in this paper were the patients' age group (in years), sex, marital status, ART duration (in years), TB screening, hepatitis B screening, ART adherence and ART status, while the response variable was the patients' viral load levels. In this paper, the patients' viral load levels was split into 3 categories, namely undetectable (viral load of at most $40\ copies/ml$), viral blips (viral load between 41 and $999\ copies/ml$) and virologic failure (viral load of at least $1000\ copies/ml$). The viral blips group was used as the reference category in the fitted multinomial logit model. All data analysis aspects of this paper were performed using the R programming language (R Core Team, 2020).

3 Results

Out of the 3047 patients (aged 15-64 years who had been on ART for at least 6 months and had a viral load measurement, TB and hepatitis B screening results) considered as per the inclusion criteria of this study, 1797 (58.98%) patients had an undetectable viral load, 639 (20.97%) had viral blips, while 611 (20.05%) had virologic failure as shown in Table 1. Out of the 3013 patients with good adherence to their ART, 1787 (58.65%) patients had undetectable viral load, while 633 (20.78%) and 593 (19.46%) had viral blips and virologic failure respectively. Majority of the patients who had undetectable viral load had been on ART for a duration of 1-3 and 4-6 years, while majority who had virologic failure had been on ART for a duration of 7-9 years, as shown in Table 1. In addition, majority of the adult patients were still actively on ART, with 1055 patients having undetectable viral load, while 334 and 303 had viral blips and virologic failure respectively. Furthermore, majority of the patients had never been testing for hepatitis B, regardless of their viral load levels, while majority of them tested negative for TB, as shown in Table 1.

Moreover, from Table 1, with a significant p-value at a 5% level of significance, the patients' age group ($p-$value$< 2.20e^{-16}$), sex ($p-$value$= 3.40e^{-03}$), marital status ($p-$value$= 1.25e^{-08}$), ART duration ($p-$value$< 2.20e^{-16}$), TB screening ($p-$value$= 1.72e^{-06}$), hepatitis B screening ($p-$value$= 3.78e^{-04}$), ART adherence ($p-$value$= 4.84e^{-05}$) and ART status ($p-$value$= 5.48e^{-07}$) can be concluded to have a significant impact on the patients' viral load levels. Thus, all these impacting patient' characteristics were considered in the fitted multinomial logit model and the subsequent results shown in Table 2, with the viral blips category used as the reference category.

From Table 2, with a significant $p-$value of 0.003, the odds of having an undetectable viral load for ART patients who were less than 20 years old was 2.818 times higher compared to the odds of having a viral blips, while the odds of having an undetectable viral load for patients who were amid the ages of 30 to 69 years were between 0.319 to 0.625 times lower. With regards to the patients' duration on ART, the odds of having an undetectable viral load for those who had been on ART amid 4 to 15 years were between 2.138 to 3.618 times higher compared to the odds of having a viral blips, with all significant $p-$value< 0.001. At a significant $p-$value< 0.001, the odds of having an undetectable viral load for patients who were separated from their spouses and those who tested positive for TB were $8.26e^{-06}$ and $1.33e^{-05}$ times lower (respectively) compared to the odds of having a viral blips as shown in Table 2.

On the other hand, with significant $p-$value< 0.001 and 0.008, the odds of having a virologic failure for patients who had been on ART for 7-9 years and 10-12 years were 0.518 and 0.533 times lower (respectively) compared to the odds of having a viral blips, while the odds of having a virologic failure for patients who had been on ART for 13-15 years was 0.519 times lower as shown in Table 2. Looking at the patients' hepatitis B screening, the odds of having a virologic failure for those who had reactive hepatitis B was 0.560 times lower ($p-$value$= 0.016$) compared to the odds of having a viral blips, while the odds of having a virologic failure for patients who were never tested for hepatitis B was 1.415 times higher ($p-$value$= 0.001$). With respect to the patients' marital status, at a significant $p-$value of 0.003 and 0.015, the odds of having a virologic failure for those who were never married and those who were widow/ers were 0.600 and 0.645 times lower (respectively) compared to the odds of having a viral blips. Looking at the patients' ART status and TB screening, the odds of having a virologic failure for those who were transferred outside the Nankudu health district were 0.667 times lower ($p-$value$= 0.007$) compared to the odds of having a viral blips, while the odds of having a virologic failure for patients who were never tested for TB was 0.618 times lower ($p-$value< 0.001) as shown in Table 2.

4 Discussion

In this paper, the multinomial logit regression model was used to statistically examine the impacting factors of ART adult patients' viral load levels using data collected from the Nankudu health district. Majority of the

patients who had undetectable viral load had been on ART for a duration of 1-3 and 4-6 years, while majority who had virologic failure had been on ART for a duration of 7-9 years. In addition, majority of the patients had never been testing for Hepatitis B but tested negative for TB.

From this study, it was revealed that patients' characteristics such as age group, sex, marital status, ART duration, TB screening, hepatitis B screening, ART adherence and ART status had a significant impact on the patients' viral load levels. While hepatitis B is not as fatal as HIV, HIV weakens the immune system which is supposed to protect the body from attack thereby increasing the risk of TB in people with HIV. However, the coinfections of TB and hepatitis B with HIV can be very fatal on the immune system of the affected person's body, thus, impacting their viral load levels when placed on ART. Likewise, since achieving a viral load suppression is the ultimate goal of HIV treatment, the longer the patients are on the treatment and the stronger their adherence are to the treatment, the higher the impact on their viral load levels. In addition, it is a commonly known perception that females generally seek various health services (such as antenatal care, family planning, HIV treatment, TB, etc.) compared to their male counterparts. If treatment is not started for an HIV patient due to a number of behavioural and psychosocial factors such as peer pressure, anxiety, rejection of diagnosis, stigma and cultural beliefs, the likelihood of having a virologic failure would be higher which could lead to the development of opportunistic infections and AIDS-related death for such patient. This study key finding is similar to the conclusions made in Di Mascio *et al.* (2004); Jobanputra *et al.* (2015); Bezabhe *et al.* (2016) and Ali and Yirtaw (2019). Bezabhe *et al.* (2016) and Jobanputra *et al.* (2015) concluded that HIV treatment adherence is robustly associated with viral load outcomes based on the various measures of adherence, although receiving treatment adherence counselling was not associated with higher likelihood of viral load suppression (Jobanputra *et al.*, 2015). Ali and Yirtaw (2019) concluded that marital status had an association with viral load outcome, while in Di Mascio *et al.* (2004), it was concluded that the duration on HIV treatment had an association with viral load outcome.

Moreover, ART patients who were less than 20 years old were more likely to have an undetectable viral load, while those between the ages of 30 to 69 years were less likely to. This is not surprising as younger patients are still under the care of their parents/guardians/caregivers who are the authoritative figures in their lives and would most often ensure constant monitoring of and adherence to their ART treatment. On the other hand, the older the patients gets, the more prone they are to contacting other adult-related health illnesses or diseases such as diabetes, high blood pressure and hypertension (to mention a few) thereby affecting their immune system that is already weakened by HIV. Additionally, patients who were never married and those who were widow/ers were less likely to have a virologic failure, while those who were separated from their spouses were less likely to have an undetectable viral load. This is not surprising as a lot of households in Namibia, especially in the Kavango West region, are often headed by single/unmarried persons who are (every so often) the breadwinners of their respective households, thereby contributing to their need to adhere to their ART treatment due to low social economic status. Both of these study key findings somewhat echo the observations made by Kiweewa *et al.* (2019) that patients with younger age and single (unmarried) marital status were associated with higher likelihood of viral load suppression. Likewise, patients who had been on ART amid 4 to 15 years were more likely to have an undetectable viral load, while those who had been on ART amid 7 to 15 years were less likely to have a virologic failure. These findings are in line with findings reported by Di Mascio *et al.* (2004) where it was concluded that higher likelihood of viral load suppression was associated with the duration on HIV treatment. ART Patients who had reactive hepatitis B and were never tested for TB were less likely to have a virologic failure, while patients who were never tested for hepatitis B were more likely to have a virologic failure and those who tested positive for TB were less likely to have an undetectable viral load. Although hepatitis B is not as fatal as HIV while the risk of TB in people with HIV is increased by HIV, their coinfections with HIV can be very fatal on the immune system of the affected person's body. As a result, the likelihood of achieving an undetectable viral load for such ART patient who have never been tested for hepatitis B and/or TB is lower.

5 Conclusion

In conclusion, it is recommended that the Namibian government and policy makers consider making hepatitis B screening mandatory, especially, among HIV patients and ART patients. Moreover, due to poor viral load monitoring at most health centres in the Nankudu health district, there is a need to intensify constant viral load monitoring to avoid (rapid) progression of patients from viral blips to virologic failure. Additionally, periodic viral load testing to monitor responses to ART is important and needs to be conducted to prevent progression of patients to virologic failure and drug resistance.

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Declaration of conflict of Interest

The authors declare that there are no conflicts of interest.

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Table 1: Distribution of patients' characteristics and their viral load levels

Characteristics	Viral load levels				$p-$value
	Viral blips	Undetectable	Virologic failure		
	Count (%)	Count (%)	Count (%)	Total (%)	
ART adherence					
Fair	3 (0.098)	8 (0.263)	11 (0.361)	22 (0.722)	$4.84e^{-05*}$
Good	633 (20.775)	1787 (58.648)	593 (19.462)	3013 (98.884)	
Poor	3 (0.098)	2 (0.066)	7 (0.230)	12 (0.394)	
Total	639 (20.971)	1797 (58.976)	611 (20.053)	3047 (100)	
Age group					
< 20	16 (0.525)	31 (1.017)	69 (2.265)	116 (3.807)	$< 2.20e^{-16*}$
20-29	70 (2.297)	332 (10.896)	71 (2.330)	473 (15.523)	
30-39	225 (7.384)	639 (20.971)	191 (6.268)	1055 (34.624)	
40-49	218 (7.155)	549 (18.018)	191 (6.268)	958 (31.441)	
50-59	94 (3.085)	214 (7.023)	80 (2.626)	388 (12.734)	
60-69	16 (0.525)	32 (1.050)	9 (0.295)	57 (1.871)	
Total	639 (20.971)	1797 (58.976)	611 (20.053)	3047 (100)	
ART duration					
1-3	125 (4.102)	573 (18.805)	57 (1.871)	755 (24.778)	$< 2.20e^{-16*}$
4-6	158 (5.185)	531 (17.427)	127 (4.168)	816 (26.780)	
7-9	194 (6.367)	374 (12.274)	221 (7.253)	789 (25.894)	
10-12	127 (4.168)	251 (8.238)	166 (5.448)	544 (17.854)	
13-15	35 (1.149)	68 (2.232)	40 (1.313)	143 (4.693)	
Total	639 (20.971)	1797 (58.976)	611 (20.053)	3047 (100)	
Hepatitis B screening					
Non-reactive	200 (6.564)	459 (15.064)	170 (5.579)	829 (27.207)	$3.78e^{-04*}$
Reactive	36 (1.181)	52 (1.707)	22 (0.722)	110 (3.610)	
Not tested	403 (13.226)	1286 (42.205)	419 (13.751)	2108 (69.183)	
Total	639 (20.971)	1797 (58.976)	611 (20.053)	3047 (100)	

Marital status					
Cohabiting	59 (1.936)	286 (9.386)	49 (1.608)	394 (12.931)	$1.25e^{-08*}$
Divorced	2 (0.066)	6 (0.197)	3 (0.098)	11 (0.361)	
Married	157 (5.153)	407 (13.357)	125 (4.102)	689 (22.612)	
Never married	260 (8.533)	626 (20.545)	298 (9.780)	1184 (38.858)	
Separated	1 (0.033)	4 (0.131)	0 (0.000)	5 (0.164)	
Widow/er	9 (0.295)	30 (0.985)	11 (0.361)	50 (1.641)	
Unknown	151 (4.956)	438 (14.375)	125 (4.102)	714 (23.433)	
Total	639 (20.971)	1797 (58.976)	611 (20.053)	3047 (100)	
Sex					
Female	460 (15.097)	1345 (44.142)	415 (13.620)	2220 (72.859)	$3.40e^{-03*}$
Male	179 (5.875)	452 (14.834)	196 (6.433)	827 (27.141)	
Total	639 (20.971)	1797 (58.976)	611 (20.053)	3047 (100)	
ART status					
Active	334 (10.962)	1055 (34.624)	303 (9.944)	1692 (55.530)	$5.48e^{-07*}$
Lost to follow up	54 (1.772)	147 (4.824)	39 (1.280)	240 (7.877)	
Pre-lost to follow up	145 (4.759)	414 (13.587)	157 (5.153)	716 (23.499)	
Stopped ART	2 (0.066)	2 (0.066)	1 (0.033)	5 (0.164)	
Transferred out	104 (3.413)	179 (5.875)	111 (3.643)	394 (12.931)	
Total	639 (20.971)	1797 (58.976)	611 (20.053)	3047 (100)	
TB screening					
Negative	490 (16.081)	1520 (49.885)	479 (15.720)	2489 (81.687)	$1.72e^{-06*}$
Positive	1 (0.033)	9 (0.295)	0 (0.000)	10 (0.328)	
Not tested	148 (4.857)	268 (8.796)	132 (4.332)	548 (17.985)	
Total	639 (20.971)	1797 (58.976)	611 (20.053)	3047 (100)	

* Significant at a 5% level of significance

Table 2: Output from the fitted multinomial logit model

	Virologic failure			Undetectable		
Characteristic	Adjusted estimate	Odd ratio	P-value	Adjusted estimate	Odd ratio	P-value
(Intercept)	1.991	7.321	0.006*	0.472	1.603	0.518
ART adherence						
Fair (ref)						
Good	-0.025	0.975	0.971	-1.097	0.334	0.103
Poor	-1.252	0.286	0.318	0.385	1.469	0.711
Age group						
20-29 (ref)						
30-39	-0.296	0.744	0.067	-0.470	0.625	0.023*
40-49	-0.252	0.778	0.146	-0.622	0.537	0.005*
50-59	-0.375	0.687	0.066	-0.709	0.492	0.005*
60-69	-0.604	0.547	0.088	-1.142	0.319	0.016*
< 20	-0.504	0.604	0.150	1.036	2.818	0.003*
ART duration						
1-3 (ref)						
4-6	-0.140	0.869	0.329	0.760	2.138	$< 0.001*$
7-9	-0.658	0.518	$< 0.001*$	1.144	3.138	$< 0.001*$
10-12	-0.630	0.533	$< 0.001*$	1.191	3.289	$< 0.001*$
13-15	-0.656	0.519	0.008*	1.286	3.618	$< 0.001*$
Hepatitis B screening						
Non-reactive (ref)						
Not tested	0.347	1.415	0.001*	0.234	1.264	0.075
Reactive	-0.580	0.560	0.016*	-0.327	0.721	0.278
Marital status						
Cohabiting (ref)						
Divorced	-0.373	0.689	0.658	0.585	1.795	0.536
Married	-0.310	0.733	0.088	-0.282	0.754	0.238
Never married	-0.510	0.600	0.003*	-0.031	0.969	0.888
Separated	0.507	1.660	0.658	-11.705	$8.26e^{-06}$	$< 0.001*$
Unknown	-0.110	0.896	0.794	0.409	1.505	0.418
Widow/er	-0.439	0.645	0.015*	-0.318	0.728	0.188
Sex						
Female (ref)						
Male	-0.138	0.871	0.217	0.223	1.250	0.098
ART status						
Active (ref)						
Lost to follow up	0.014	1.014	0.938	-0.228	0.796	0.327
Pre-lost to follow up	0.067	1.069	0.597	0.232	1.261	0.133
Stopped ART	-0.348	0.706	0.779	-1.518	0.219	0.283
Transferred out	-0.405	0.667	0.007*	0.209	1.233	0.226
TB screening						
Negative (ref)						
Not tested	-0.482	0.618	$< 0.001*$	-0.303	0.739	0.051
Positive	0.911	2.486	0.392	-11.225	$1.33e^{-05}$	$< 0.001*$

* Significant at a 5% level of significance (ref) = reference category