Research Article

Species, Risk Factors, and Antimicrobial Susceptibility Profiles of Bacterial Isolates from HIV-Infected Patients Suspected to Have Pneumonia in Mekelle Zone, Tigray, Northern Ethiopia

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Received 12 February 2019; Revised 2 April 2019; Accepted 16 April 2019; Published 5 May 2019

Academic Editor: Lucia Lopalco

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Background. Pneumonia is a condition, where bacterial infections are implicated as the most common causes of morbidity and mortality in humans. The actual burden of HIV-infected patients with pneumonia is not well documented in Mekelle region of Ethiopia. This study estimated the prevalence of bacterial pneumonia in HIV patients, antimicrobial susceptibility patterns of pathogens implicated in pneumonia, and associated risk factors in Mekelle zone, Tigray, Northern Ethiopia, during August-December 2016. Methods. Sputum specimens were collected from 252 HIV seropositive individuals with suspected pneumonia. Data on sociodemographics and risk factors were also collected using a structured questionnaire. Blood, Chocolate, and Mac Conkey agar plates (Oxoid, Hampshire, UK) were used to grow the isolates. The isolated colonies were identified based on Gram stain, colony morphology, pigmentation, hemolysis, and biochemical tests. The antimicrobial susceptibility test was performed using the modified Kirby-Bauer disc diffusion method. The analysis was performed using SPSS version 22 and p-value < 0.05 with corresponding 95% confidence interval (CI) was considered statistically significant. Results. Out of the 252 samples, 110 (43.7%) were positive for various bacterial species. The predominant bacterial species were Klebsiella pneumoniae (n=26, 23.6%) followed by Streptococcus pneumoniae (n=17, 15.5%), Escherichia coli (n=16, 14.5%), Klebsiella spp. (n=15, 13.6%), Staphylococcus aureus (n=9, 8.2%), Enterobacter spp. (n=7, 6.3%), Pseudomonas aeruginosa (n=4, 3.6%), Proteus spp. (n=4, 3.6%), Citrobacter freundii (n=7, 6.3%), Streptococcus pyogenes (n=3, 2.7%), and Haemophilus influenzae (n=2, 1.8%). Young age (18-29), recent CD4+ count less than 350 cells/mL, alcohol consumption, and HIV WHO stage II showed significant association with the occurrence of bacterial pneumonia. Resistance to penicillin, co-trimoxazole, and tetracycline was observed in 81.8%, 39.8%, and 24.5% of the isolates, respectively. Conclusions. The problem of pneumonia among HIV patients was significant in the study area. The high prevalence of drug-resistant bacteria isolated from the patient’s samples possesses a health risk in immunocompromised HIV patients. There is a need to strengthen and expand culture and susceptibility procedures for the administration of appropriate therapy to improve patients management and care which may aid in decreasing the mortality.

1. Background

Respiratory infections are the leading cause of morbidity and mortality in HIV-infected individuals [1]. It is also the most frequent cause of hospital admission in HIV-infected people worldwide with an incidence of 20-25 episodes per 100 hospital admissions per year [2]. About 70% of HIV patients have a pulmonary complication during the evolution of the disease, mainly as a consequence of infectious etiology [2]. During respiration, the respiratory tract, especially nose,
nasopharynx, and oropharynx, comes into contact with a large number of bacteria that may cause life-threatening respiratory infections [3]. Lower respiratory tract infections (LRTIs) are among the most prevalent diseases of human beings worldwide [4], which result in around 7 million deaths annually [5]. Pneumonia is an acute respiratory illness which is one of the most common LRTI, mainly caused by bacteria, viruses, and fungi [6, 7].

Bacterial pneumonia is the most frequent cause of pulmonary infections, which is among the most common causes of respiratory failure in HIV-infected patients [2, 8]. The incidence of bacterial pneumonia in HIV-infected patients ranges from 1.93 to 19.2% of cases per year in Nigeria [9]. HIV-infected individuals have a sixfold greater risk of bacterial pneumonia than the noninfected individuals [10] and estimated greater than one-third of all persons with AIDS will develop at least one episode of severe bacterial pneumonia over the course of their HIV infection [10]. If bacterial pneumonia is not properly treated, it causes further complications leading to empyema, meningitis, pericarditis, hepatitis, and arthritis [10].

The most common pathogens that cause bacterial pneumonia are S. pneumoniae, H. influenzae, K. pneumoniae, P. aeruginosa, E. coli, and S. aureus [11]. Among this S. pneumoniae is considered the predominant bacterial pathogen across all age groups and accounts for approximately 30% of pneumonia cases [12].

The incidence of bacterial drug resistance is high due to empirical drug administration and poor adherence to treatment standards. Hence, specific antibiotic therapy is fundamental to manage and control bacterial pneumonia in HIV patients [13]. Therefore, identifying the bacterial cause and performing antibiotic susceptibility testing are vital [2].

In Northern Ethiopia, there is a high prevalence of HIV-infected patients [14]. However, there is no study which shows the real burden of bacterial pneumonia among HIV positive individuals in this region. In addition, the antimicrobial susceptibility patterns of the common bacterial agents for pneumonia are not known. Therefore, this study was performed to assess the burden of bacterial pneumonia, the risk factors, and antimicrobial susceptibility of bacterial isolates in HIV positive individuals with suspected pneumonia at ART clinics of Mekelle zone, Tigray, Northern Ethiopia.

2. Methods

2.1. Study Design, Specimen Collection, and Transportation. A multicentre cross-sectional study was conducted from August-December 2016 at different ART clinics of Mekelle zone, Tigray, Ethiopia. A total of 252 HIV patients with suspected pneumonia were included in the study based on the inclusion/exclusion criteria. Sociodemographic, clinical, and risk-factor related data were collected using a structured and pretested questionnaire. Pneumonia suspected individuals showing clinical symptoms such as shortness of breath, chest pain, fever, chills, tiredness, and cough were examined by clinicians and the sputum samples were collected and transported to medical microbiology laboratory, Mekelle University, College of Health Sciences by medical laboratory technologists for analysis within 2 hrs or stored at 4°C until further processing.

2.2. Inclusion and Exclusion Criteria. HIV seropositive, highly active antiretroviral therapy users and nonusers aged ≥ 18 with a diagnosis of pneumonia as described by the clinician were included. Patients who were critically ill, who cannot produce sputum, suspected pulmonary tuberculosis and who took antibiotics during the past two week period were excluded.

2.3. Specimen Processing. Smear was prepared from each sputum sample for Gram staining and slides were examined under low power field (10X). Sputum samples with > 25 polymorphonuclear leukocytes and <10 epithelial cells were accepted for culture. Samples that had more than 10 epithelial cells and less than 25 polymorphonuclear leukocytes per low power field were discarded [15, 16].

2.4. Cultivation and Identification of Isolates. Blood, Chocolate, and Mac Conkey agar plates (Oxoid, Hampshire, UK) were prepared as per the manufacturers’ instructions. Using a calibrated wire loop, approximately 0.1μL purulent sputum sample was streaked onto each agar plate. Chocolate agar plates were incubated at 37°C for 24 hours in a candle jar. MacConkey agar and blood agar plates were incubated aerobically at 37°C for 24 hours [15, 16]. After incubation, the plates were inspected for any growth and negative plates were incubated for an additional 24 hours. Quantitative sputum culture analysis was carried out and the significant bacterial count was reported on observing an excess of 10^6 CFU/mL [17]. More than 100 CFU were considered significant for bacterial growth in this study. A single pure culture colony of each isolate was stored in nutrient broth with 10% glycerol stock solution for identification. Gram-positive cocci were differentiated and identified based on Gram stain, patterns of hemolysis on blood agar, colonial characteristics, catalase test, coagulase test, optochin susceptibility, and bacitracin susceptibility. Gram-negative bacteria were also identified based on Gram stain, colonial morphology and pigmentation, oxidase test, carbohydrate fermentation, H2S production, citrate utilization, motility, indole formation, urea hydrolysis and growth in blood agar with “X”, and “V” factors for H. influenzae.

2.5. Antimicrobial Susceptibility Testing. The antimicrobial susceptibility test was performed using the Kirby-Bauer disc diffusion test method [16]. The antimicrobial discs used for susceptibility testing were amikacin (AK,30μg), ciprofloxacin (CIP,5μg), ceftriaxone (CTR,30μg), chloramphenicol (C,10μg), co-trimoxazole (COT,25μg), erythromycin (ERY,15μg), gentamicin (GN,10μg), Methicillin (MET, 30μg) penicillin (P,10 units), and tetracycline (TE,30 μg). Muller-Hinton agar (MHA) plates were used for the nonfastidious organism, whereas MHA supplemented with 5% sheep blood were used for S. pneumoniae and Haemophilus spp. using a standard procedure [16]. The results were interpreted using the guidelines described by the CLSI (2016) standards. Multidrug resistance was defined by nonsusceptibility to at least one
agent in three or more antimicrobial categories as described earlier by Magiorakos et al. [18].

2.6. Quality Assurance. The training was provided to data collectors on how to perform the questionnaire and sampling processing. The filled questionnaires were checked for their completeness. Media preparations were made based on the manufacturer’s instruction. Quality control slides were incorporated during Gram staining. Standard operating procedures were followed during specimen collection, handling, transportation, microbiological analysis, and interpretation. Five percent of media per batch were incubated overnight for sterility check. Reference strains S. aureus ATCC25923, E. coli ATCC25922, and P. aeruginosa ATCC27853 were used during cultivation and antimicrobial susceptibility testing [16].

2.7. Statistical Analysis. Data was analyzed using SPSS version 22, and descriptive statistics, bivariate, and multivariate logistic regression were employed. A bivariate logistic regression model was used to determine the possible association of each variable with the outcome variable and multivariate analysis was done to those independent variables with p-value < 0.05 to identify factors that are independently associated with the dependent variable. P-value <0.05 with 95% confidence interval was considered as statistically significant.

3. Results

3.1. Sociodemographic Characteristics. This study was conducted among 252 study participants. Out of 224 (88.9%) were urban dwellers and 28 (11.1%) were rural dwellers. The mean age of study participants was 40.58 ± 9.9, range 18-67 years old, and 50% being male and the rest female (Table 1).

3.2. Prevalence of Bacterial Pneumonia. Bacterial pneumonia was observed in 110 (43.7 %) and out of those 59 (46.6 %) of them were male participants (Table 2). Bacterial pneumonia was found highest in age group 18-29 (n=15, 50 %) and lowest in the age group 50-67, 14 (28.6%). Out of the 110 isolates, 95 (42.4 %) were isolated from urban dwellers. The predominant bacterial species were Klebsiella pneumoniae (n=26, 23.6%) followed by Streptococcus pneumoniae (n=17, 15.5 %), Escherichia coli (n=16, 14.5%), Klebsiella spp. (n=15, 13.6%), Staphylococcus aureus (n=9, 8.2%), Enterobacter spp. (n=7, 6.3%), Pseudomonas aeruginosa (4, n=3.6%), Proteus spp. (n=4, 3.6%), Citrobacter freundii (n=7, 6.3%), Streptococcus pyogenes (3, 2.7%), and Haemophilus influenzae (n=2, 1.8%).

3.3. Antimicrobial Susceptibility Pattern of Bacterial Isolates. Out of the tested antibiotics, 39.8% (n=40 isolates) were resistant to co-trimoxazole and 24.5% (n=26) for tetracycline. In this study, 81.8% (n=9) Gram-positive isolates and 27.6% (n=8) were resistant to penicillin and erythromycin, respectively (Table 3). Similarly, 10.7% (n=8) of Gram-negative isolates were resistant to ceftriaxone (Table 3). On the other hand, most of the isolates were less resistant to amikacin (n=6, 6.8%) and ciprofloxacin (n=8, 9.1%). Methicillin resistance was observed in 44.4% (n=4) isolates of S. aureus (Table 3).

| Variables | Frequency | Percent (%) |
|-----------|-----------|-------------|
| Gender    |           |             |
| Male      | 127       | 50.4        |
| Female    | 125       | 49.6        |
| Age (in years) |       |             |
| 18-29     | 30        | 11.9        |
| 30-39     | 98        | 38.9        |
| 40-49     | 75        | 29.8        |
| 50-67     | 49        | 19.4        |
| Residence |           |             |
| Urban     | 224       | 88.9        |
| Rural     | 28        | 11.1        |
| Occupation|           |             |
| Farmer    | 19        | 7.5         |
| Employed  | 78        | 31          |
| Unemployed| 110       | 43.6        |
| Self-employed | 45  | 17.9       |
| Educational level | |            |
| Illiterate | 42       | 16.7        |
| Read and write | 33      | 13.1        |
| 1-8       | 80        | 31.7        |
| 9-12      | 59        | 23.4        |
| College and above | 38 | 15.1       |

Multidrug resistance was observed in 18% (n=15) of isolates (Table 4).

3.4. Associated Risk Factors. All variables with a significance value in the bivariate analysis were entered in to multiple logistic regression model and in multivariate analysis the variables age groups 18-29 (AOR = 3.8, 95% CI: 1.25-11.52, P ≤ 0.018), age groups 30-39 (AOR = 3.4, 95% CI: 1.37-8.25, P ≤ 0.008), recent number of CD4+ cell count less than 350 (AOR = 2.4, 95% CI:1.11-5.09, P ≤ 0.026), alcohol consumption (AOR=7.4, 95% CI: 2.46-22.11, P ≤ 0.001), and WHO stage of HIV infection (AOR= 5.4, 95% CI: 2.00-14.36, P ≤ 0.001) were found to have statistically significant association with bacterial pneumonia. In the bivariate analysis, gender and occupation failed to show a significant association with the occurrence of bacterial pneumonia. Although not statistically significant, bacterial pneumonia was higher in cigarette smokers, in non-cART users, and in those who interrupt cART (Table 5).

4. Discussion

In this study, the overall prevalence of bacterial pneumonia was found to be 43.7%. This is in line with the studies reported from Nigeria 42.9 % [19] and India 44.3 % [20]. This is due to the fact that individuals with compromised immune status are more susceptible to bacterial infections. On the other hand, our finding is higher than the findings from Malawi 29
Table 2: Rate of bacterial pneumonia with regard to the sociodemographic characteristics among pneumonia suspected HIV patients in ART clinics of Mekelle zone, Northern Ethiopia, August 2016-December 2016.

| Variables          | Positive N (%) | Bacterial occurrence | Negative N (%) | Total |
|--------------------|----------------|----------------------|----------------|-------|
| Gender             |                |                      |                |       |
| Male               | 59 (46.6)      |                      | 68 (53.5)      | 127   |
| Female             | 51 (40.8)      |                      | 74 (59.2)      | 125   |
| Age (in years)     |                |                      |                |       |
| 18-29              | 15 (50)        |                      | 15 (50)        | 30    |
| 30-39              | 47 (48)        |                      | 51 (52)        | 98    |
| 40-49              | 34 (45.3)      |                      | 41 (54.7)      | 75    |
| 50-67              | 14 (28.6)      |                      | 35 (71.4)      | 49    |
| Residence          |                |                      |                |       |
| Urban              | 95 (42.4)      |                      | 129 (57.6)     | 224   |
| Rural              | 15 (53.6)      |                      | 13 (46.4)      | 28    |
| Occupation         |                |                      |                |       |
| Farmer             | 9 (47.4)       |                      | 10 (52.6)      | 19    |
| Employed           | 32 (41)        |                      | 46 (59)        | 78    |
| Unemployed         | 46 (41.8)      |                      | 64 (58.2)      | 110   |
| Self-employed      | 23 (51.1)      |                      | 22 (48.9)      | 45    |
| Educational level  |                |                      |                |       |
| Illiterate         | 14 (33.3)      |                      | 28 (66.7)      | 42    |
| Read and write     | 10 (30.3)      |                      | 23 (69.7)      | 33    |
| 1-8                | 37 (46.3)      |                      | 43 (53.7)      | 80    |
| 9-12               | 30 (50.8)      |                      | 29 (49.2)      | 59    |
| College and above  | 19 (50)        |                      | 19 (50)        | 38    |

% [13] and India 18.4 % [21] and lower than the study reported from Nigeria 55.6% [9]. The inconsistency may be due to sampling size variation, type of sample used, and immune status of the study participants.

In our study, K. pneumoniae and S. pneumoniae were the predominant pathogens isolated as reported in similar studies from Nepal [22, 23] and India [20]. On the other hand, different predominant isolates, such as E. coli and P. aeruginosa were reported from Nigeria [9] while S. pneumoniae and H. influenzae were reported from Uganda as dominant bacterial species [24]. A climatic and geographic variation could be the possible reasons for the different frequency of the bacterial isolates in different study area among HIV patients.

E. coli was the second most common Gram-negative bacterium to be isolated in our study; which in agreement with the study reported from Bahrain [25] and Nigeria [9]. Among the Gram-positives S. aureus was the second most common species to be isolated; this is consistent with a study reported from the United States [26], Italy [27], and Greece [28]. The occurrence might be due to aspiration of the bacteria from the previously colonized site, prior hospitalization, contaminated foods eaten, and mostly systemic spread from blood to the lungs in these patients [26–28].

WHO recommends co-trimoxazole as an intervention to reduce infections by opportunistic pathogens including bacterial pneumonia in HIV positive patients. In this study, about 39.8% and 24.5% of bacterial isolates were resistant to co-trimoxazole and tetracycline. This is similar to the study reported in Nigeria [9] and Tanzania [20]. This may be due to the consistent use of this antibiotic, which might contribute to the development of drug resistance. Out of the tested Gram-positive isolates, 81.8 % and 27.6 % were resistant to penicillin and erythromycin respectively. This might be because these antibiotics have been very commonly prescribed for many years during the course of infection.

According to the international standard for the definition of drug resistance [18], 17.9% isolates were multidrug resistant. We did not have data from our study participants engaging in self-medication but empirical treatment still is a contributing factor for the emergence of drug resistance in these patients [9, 29]. Out of the S. aureus isolates, 44.4 % were methicillin resistance but because the sample size was low these results may not be significant. This is higher from the study reported from Bahrain, 10.5% [25], and lower from Italy, 65% [27] in this study, age groups 18-29 and 30-39, alcohol consumption, recent CD4+ count less than 350 cells/μL, and WHO stage II of HIV infection showed significant association to have bacterial pneumonia. Age groups, 18-29 and 30-39, were 3.8 and 3.4 times more likely to have bacterial pneumonia, respectively, compared to the age group, 50-67 which is in agreement with studies reported from Nigeria [9] and Nepal [22]. The reason may be due to the fact that a larger number of younger age groups of our study participants were within CD4+ cell count less than 350 cells/μL and WHO stage II compared to those aged between 50 and 67. This
Table 3: Antimicrobial susceptibility patterns of bacterial isolates among pneumonia suspected HIV patients in ART clinics of Mekelle zone, Northern Ethiopia, August-December 2016.

| Isolates (n)       | GN  | AK  | CTR | P  | CIP | COT | ERY | C  | TE | MET |
|--------------------|-----|-----|-----|----|-----|-----|-----|----|----|-----|
| S. pneumoniae (17) | S   | NT  | NT  | NT | NT  | NT  | 6(35.3) | 1(16.7) | 14(82.4) | 10(58.8) | NT |
|                    | R   | NT  | NT  | NT | NT  | NT  | NT  | NT | NT | NT  |
| S. aureus (9)      | S   | 8(88.9) | 9(100) | NT | 2(22.2) | 8(88.9) | 6(66.7) | 6(66.7) | 7(77.8) | 9(100) | 5(55.6) |
|                    | R   | 1(11.1) | 0 | 7(77.8) | 1(11.1) | 3(33.3) | 2(22.2) | 0 | 2(22.2) | 4(44.4) |
| S. pyogenes (3)    | S   | NT  | NT  | NT | 1(33.3) | NT  | 3(100) | NT | NT | NT  |
| K. pneumoniae (26) | S   | 21(80.8) | 23(88.5) | 24(92.3) | NT  | 26(100) | 15(57.7) | NT | 23(88.5) | 19(73.1) | NT |
|                    | R   | 5(19.2) | 3(11.5) | 2(7.7) | 0 | 11(42.3) | NT | 3(11.5) | 7(26.9) |
| E. coli (16)       | S   | 14(87.5) | 15(93.8) | 13(81.3) | NT  | 14(87.5) | 10(62.5) | NT | 16(100) | 15(93.8) | NT |
|                    | R   | 2(12.5) | 1(6.2) | 3(18.7) | 2(12.5) | 6(37.5) | NT | 0 | 1(6.2) |
| Klebsiella spp. (15)| S  | 14(93.3) | 14(93.3) | 14(93.3) | NT  | 14(93.3) | 14(93.3) | NT | 15(100) | 14(93.3) | NT |
|                    | R   | 1(6.7) | 1(6.7) | 1(6.7) | 1(6.7) | NT | NT | 0 | 1(6.7) |
| Enterobacter spp. (7) | S  | 7(100) | 7(100) | 7(100) | NT  | 3(42.9) | 3(42.9) | NT | 3(42.9) | 7(100) | NT |
|                    | R   | 0 | 0 | 0 | 0 | 4(57.1) | 4(57.1) | NT | 4(57.1) | 0 |
| Proteus spp (4)    | S   | 4(100) | 3(75) | 4(100) | NT  | 4(100) | 4(100) | NT | 3(75) | 3(75) | NT |
|                    | R   | 0 | 0 | 0 | 0 | 4(57.1) | 4(57.1) | NT | 4(57.1) | 0 |
| Citrobacter freundii (7) | S  | 7(100) | 7(100) | 7(100) | NT  | 7(100) | 5(71.4) | NT | 5(71.4) | 5(71.4) | NT |
|                    | R   | 0 | 0 | 0 | 0 | 2(28.6) | 2(28.6) | NT | 2(28.6) | 2(28.6) |
| P. aeruginosa (4)  | S   | 3(75) | 4(100) | NT  | NT | 4(100) | NT  | NT | NT | NT  |
|                    | R   | 1(25) | 0 | 0 | 0 | NT | NT | NT | NT | NT  |
| H. influenzae (2)  | S   | NT  | NT  | NT  | NT | NT  | 2(100) | 0 | 2(100) | 0 |
|                    | R   | 10(11.4) | 6(8.6) | 9(11.7) | 8(9.1) | 40(39.8) | 8(27.6) | 15(14.2) | 26(24.5) | 4(44.4) |

NT= not tested, Klebsiella spp. = other Klebsiella species. P = penicillin, CTR= ceftriaxone, GN = gentamicin, AK= amikacin, COT= co-trimoxazole, TE = tetracycline, C = chloramphenicol, CIP = ciprofloxacin, MET= methicillin, ERY = erythromycin, S=susceptible, and R= resistant. % is computed from each cell. Total= total calculated from both Gram negative and positive isolates for that specific antibiotics were tested.
In this study, although not statistically significant, bacterial pneumonia was higher among cigarette smokers. Smoking lowers the action and number of cilia and inhibits the ability to prevent the entry of microorganisms to the respiratory tract. This is supported by studies reported earlier which indicate cigarette smoking increases the risk of bacterial pneumonia two times compared to nonsmokers [35].

5. Conclusions

In this study, the prevalence of bacterial pneumonia was 43.7 % caused majorly by Gram-negative bacterial species such as \textit{K. pneumoniae} and \textit{E. coli}. In Gram-positive isolates, \textit{S. pneumoniae} and \textit{S. aureus} were the most dominant species of bacteria. Most isolates were susceptible to amikacin, ciprofloxacin, and ceftriaxone. Younger age group, the recent number of CD4+ cell count less than 350 cells, alcohol consumption, and WHO stage II of HIV infection showed significant association with bacterial pneumonia. Identification of the etiological agent and performing antimicrobial susceptibility testing in order to select an appropriate antimicrobial agent will be important in the management of bacterial pneumonia and limiting the evolution of resistant bacteria in the clinical setting.

**Abbreviations**

AIDS: Acquired Immune-Deficiency Syndrome  
AOR: Adjusted Odds Ratio  
ART: Antiretroviral Therapy  
ATCC: American Type Culture Collection  
CI: Confidence interval  
CLSI: Clinical and Laboratory Standards Institute  
COR: Crude Odds Ratio  
HIV: Human Immunodeficiency Virus  
MDR: Multidrug Resistance  
SPSS: Statistical Package for Social Sciences.
Table 5: Bivariate and multivariate logistic regression analysis of factors associated with bacterial pneumonia in pneumonia suspected HIV patients in ART clinics of Mekelle zone, Northern Ethiopia, August-December 2016.

| Variables | Bacterial pneumonia | COR (95% CI) | P-value | AOR (95% CI) | P-value |
|-----------|----------------------|--------------|---------|--------------|---------|
|           | Positive N (%)       | Negative N (%) |         |              |         |
| Gender    |                      |              |         |              |         |
| Male      | 59 (46.6)            | 68 (53.5)    | 1.3 (0.76-2.07) | 0.366 |
| Female    | 51 (40.8)            | 74 (59.2)    | 1        |              |         |
| Age (in years) |          |              |         |              |         |
| 18-29     | 15 (50)              | 15 (50)      | 2.5 (0.97-6.44) * | 0.058 |
| 30-39     | 47 (48)              | 51 (52)      | 2.3 (1.10-4.81) * | 0.026 |
| 40-49     | 34 (45.3)            | 41 (54.7)    | 2.1 (0.96-4.47)  | 0.063 |
| 50-67     | 14 (28.6)            | 35 (71.4)    | 1        |              |         |
| Occupation |                      |              |         |              |         |
| Employed  | 32 (41)              | 46 (59)      | 1        |              |         |
| Farmer    | 9 (47.4)             | 10 (52.6)    | 1.1 (0.35-3.72) | 0.825 |
| Unemployed| 46 (40)              | 64 (60)      | 0.9 (0.30-2.66) | 0.849 |
| Self-employed | 23 (51.1)  | 22 (48.9)    | 0.6 (0.31-1.35) | 0.248 |
| Recent CD4+ cells/µl |        |              |         |              |         |
| <350      | 56 (57.7)            | 41 (42.3)    | 3.3 (1.7-6.34) * | 0.001 |
| 350-500   | 34 (39.1)            | 53 (60.9)    | 1.5 (0.78-3.03) | 0.211 |
| >500      | 20 (29.4)            | 48 (70.6)    | 1        |              |         |
| Cigarette smoking |        |              |         |              |         |
| Yes       | 13 (65)              | 7 (35)       | 2.6 (0.99-6.72) | 0.051 |
| No        | 97 (41.8)            | 135 (58.2)   | 1        |              |         |
| Use cART  |                      |              |         |              |         |
| Yes       | 100 (41.8)           | 139 (58.2)   | 1        |              |         |
| No        | 10 (76.9)            | 3 (23.1)     | 4.6 (1.24-17.27) | 0.022 |
| Ever interrupt cART |        |              |         |              |         |
| Yes       | 16 (69.6)            | 7 (30.4)     | 3.6 (1.43-9.17) * | 0.007 |
| No        | 84 (38.7)            | 133 (61.3)   | 1        |              |         |
| Alcohol consumption |        |              |         |              |         |
| Yes       | 22 (81.5)            | 5 (18.5)     | 6.9 (2.5-18.76) * | 0.001 |
| No        | 88 (39.1)            | 137 (60.9)   | 1        |              |         |
| WHO Stage |                      |              |         |              |         |
| I         | 80 (38.6)            | 127 (61.4)   | 1        |              |         |
| II        | 22 (75.9)            | 7 (24.1)     | 5.0 (2.04-12.22) * | 0.001 |
| III       | 8 (50)               | 8 (50)       | 1.6 (0.57-4.40) | 0.374 |

COR=crude odds ratio; CI=confidence interval; AOR=adjusted odds ratio; 1: referent; * statically significant.

Data Availability

Data supporting the conclusions of this article are available by request from G. Adhanom. The relevant raw data will be made available to researchers wishing to use them for noncommercial purposes.

Ethical Approval

Ethical clearance was obtained from Mekelle University, College of Health Sciences, Research Ethics Review Committee. Permission was also obtained from Tigray Regional Health Bureau and institution of each ART clinics Mekelle zone.

Consent

Written informed consent was obtained from the study participants before data collection.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors’ Contributions

Muthupandian Saravanan, Gebre Adhanom, Dawit Gebregziabiher, and Yemane Weldu conceived and designed the
experiments. Gebre Adhanom, Muthupandian Saravanan, Dawit Gebreeziabiber, and Yemane Weldu performed the experiments. Muthupandian Saravanan, Gebre Adhanom, Haftom Legese, Tadele Araya, Araya Gebreyesus Wasihun, Bruno S. Lopes, and Haftom Legese analyzed the data. All authors provided a critical review of the paper.

Acknowledgments

The authors would like to acknowledge Mekelle University for financing and allowing the laboratory space and materials to conduct the laboratory work. All ART clinics of Mekelle zone and all study participants are acknowledged for their willingness to participate in this study. This work was supported by Mekelle University, College of Health Sciences, Postgraduate Students Research fund.

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