PHARYNGEAL CARRIAGE RATE AND ANTIBIOTIC SUSCEPTIBILITY PROFILE OF *NEISSERIA MENINGITIDIS* AMONG PRISONERS AT JIMMA ZONAL PRISON IN JIMMA TOWN, A CROSS-SECTIONAL STUDY, JIMMA, SOUTHWESTERN ETHIOPIA

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Jimma University
School of Medical Laboratory Science
Institute of Health Sciences

Pharyngeal Carriage Rate and Antibiotic Susceptibility Profile of *Neisseria meningitidis* Among Prisoners at Jimma Zonal Prison in Jimma Town, A Cross-Sectional Study, Jimma, Southwestern Ethiopia

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February, 2020
Jimma, Ethiopia

Declaration Sheet

I, the undersigned, MSc Medical microbiology student declares that this thesis is my original work in partial fulfillment of the requirement for the degree of master science in medical microbiology. Where others work has been used, it has been carefully acknowledged and referenced in accordance with the requirements.

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Abstract

Background

Neisseria meningitidis is known to cause severe sepsis, meningitis and pneumonia. Meningococcal carriage is a prerequisite for invasive infection and essential for transmission. This study aimed at determining the rate of pharyngeal carriage and antibiotic susceptibility pattern of meningococcal isolates among prisoners in Jimma Ethiopia.

Method

Cross-sectional study was conducted in Jimma Zonal prison in Jimma town, Southwest Ethiopia from May to October, 2019. Samples of Pharyngeal swabs were collected, processed, isolated and identified N. meningitidis using standard microbiological techniques. Colonies identified as N .meningitidis further investigated by slide-agglutination serogrouping method using antisera. Antibiotic susceptibility test were done for isolates using disk diffusion method. Data on demographic and factors for carriage were collected using a structured questionnaire and assessed using statistical methods including binary logistic regression analysis.

Result

Out of the 275 study participants (263 males and 12 females), 38(13.8%) were found carriers of N. meningitidis. The predominant isolates were Non-serogroupable 13(34.2%) and serogroup W/Y 11(28.9%) respectively. Of the isolates, 31.6% exhibited intermediate resistance and 5.3% resistance to penicillin; 15.8% of isolates exhibited resistance for ceftriaxone, in contrast, isolates were exhibited (97.4%) sensitive to chloramphenicol and 94.7% sensitive to rifampicin. Factor with increased of N. meningitidis pharyngeal carriage were being age group of 16-20 years ($p \leq 0.014$) (AOR: 5.31) 95% CI: 1.404–20.076); *having respiratory symptom within three months ($p \leq 0.048$) (AOR: 2.327)* 95% CI: 1.007-5.380), *active cigarette smoking within three months ($p \leq 0.001$) (AOR: 6.788)* 95% CI 3.007-15.326). *Antimicrobial use within three months was decrease risk of N. meningitidis pharyngeal carriage ($p \leq 0.004$) (AOR: 0. 263)* 95% CI: 0.106–0.655).

Conclusions

The participants harbour most of serogroups that are responsible for invasive cases of meningococcal disease. The isolates exhibited relatively high resistance to ceftriaxone. Respiratory symptom, active cigarette smoking and age group of 16-20 years increase risk of N. meningitidis pharyngeal carriage rate. This study suggests better to provide antibiotic prophylaxis for prisoners.

Keywords:-*Neisseria meningitides (Meningococcal), Serogroup, Prisoner, Pharyngeal, Carriage, Antibiotic susceptibility*

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Abbreviation and Acronyms

ATCC	American Type Culture Collection
BSL	Biosafety Level
CDC	Centre for Disease control
CLSI	Clinical and Laboratory Standard Institute
CTA	Cystine Trypticase Agar
IMD	Invasive Meningococcal Disease
IRB	Institutional Review Board
MD	Meningitis disease
MIC	Minimal Inhibitory Concentration
MIZ	Minimal Inhibitory Zone
MTM	Modified Thayer Martin
NG	Non-Groupable
AOR	Adjusted Odds Ratio
QC	Quality Control
USA	United State of America
VCNT	Vancomycin, Colistin, Nystatin and Trimethoprim
WHO	World Health Organization

Table of content

Page

List of figure

List of Table

1 Introduction

1.1 Background Information

Neisseria meningitidis is Gram-negative, oxidase-positive, aerobic diplococcus the family of *Neisseriaceae*, causes severe sepsis, meningitis and pneumonia(1)(2). Humans are the only natural hosts for meningococcus, and carriage in the nasopharynx, however brief, is both a prerequisite of invasive meningococcal disease (IMD) and essential for transmission(3). The main reservoir in most populations is asymptomatic pharyngeal carriage(2). The bacterium is particularly sensitive to desiccation. Transmitted from one individual to another requires close contact acquisition of *N. meningitidis* mainly through direct contact and airborne droplets inhalation(4)(5).

Neisseria meningitidis is a capsulated bacterium and is classified into serogroups based on the immunological reactivity of the capsule's polysaccharide(6)**.** Thirteen meningococcal serogroups (A, B, C, D, 29E, H, I, K, L, W135, X, Y and Z) are identified with six polysaccharide capsular serogroups (A, B, C, W135, Y, and X) are responsible for almost all invasive cases of meningococcal disease worldwide(7).

Meningococci use pili for initial attachment to the microvilli of the non-ciliated pharyngeal epithelium as a prelude to invasion; they may form part of the transient flora without producing symptom (8). Capsule is the main virulence factor and its expression undergoes genetic regulation during pathogenesis: expression needs to be down regulated or lost during carriage. On the other hand, capsule is important for survival in the blood and is consequently up regulated during invasion into the bloodstream(9). The balance between carriage of the organism and the development of the disease after acquisition is affected by *Neisseria meningitidis* characteristics such as bacterial virulence factors and also host and environmental factors including age, damage of mucosal barrier and host immune defense mechanisms(1)**.** In the invasion process, the microvilli come in close contact with the bacteria, which then enter the cells in membrane-bound vesicles(10).

Less than 1% of individuals who acquire carriage go on to develop meningococcal disease(1).Shortly after colonization, and usually less than 10 days from first exposure, meningococci can pass through the epithelial cells and enter the blood stream, where they

occasionally survive and multiply intravascular. Progression to severe meningococcal disease can occur very rapidly. The most important factors predisposing individuals to invasive meningococcal disease are the lack of circulating protective bactericidal antibodies and defects in the complement system. From the blood stream the bacterium is then disseminated to various organs and more than half of the patients developing a systemic meningococcal infection will present with clinical symptoms of meningitis(4). Meningococcal outbreaks can be started by capsular switching, which is believed to allow immunologic escape from the original serogroup. Capsular switching occurs through autologous recombination switching or acquire large DNA sequences through horizontal gene transfer from other meningococcal strains or other species(11).

Early antibiotic treatment of meningococcal disease is crucial for keeping the case fatality rate and risk of sequelae as low as possible(12). Because of *N. meningitidis* can be highly contagious, close contacts of the infected patient are treated prophylactically with rifampin, fluoroquinolone, or ceftriaxone (13).In Africa, the general recommendation for treatment during endemic periods is (i) ceftriaxone in multiple doses or (ii) multiple doses of penicillin. During meningococcal epidemics, the recommended treatment is (i) a single dose of chloramphenicol in oil or (ii) a single dose of ceftriaxone. Reduced susceptibility to penicillin, ciprofloxacin, rifampin and chloramphenicol had been reported from several countries worldwide. The resistance to penicillin mainly due to alterations in penicillin binding protein 2, encoded by the *penA* gene and resistance to chloramphenicol is considered to be due mainly to the presence of the *catP* gene, encoding the enzyme chloramphenicol acetyltransferase (12).

1.2 Statement of the Problem

Meningococcal disease is a major cause of morbidity and mortality worldwide. There are substantial cyclical fluctuations in meningococcal disease incidence and the occurrence of outbreaks and epidemics(11). Every year, it is estimated that at least 1.2 million people become sick with meningococcal disease and 135,000 individuals die worldwide(14). Meningococcal disease occurs worldwide with incidence rates varying from 1 to 1000 cases per 100,000(4**).** Meningococcal disease **(**MD) is fatal as many as 50–80% of untreated cases, and case fatality rates even in treated individuals are ~10–15%. In addition, MD causes great morbidity, with 12-20% of survivors suffering significant permanent clinical sequel (e.g. paralysis, deafness, mental impairment, amputations, and seizures), learning disabilities. However, MD is often considered as endemic globally, although epidemics occur frequently in the meningitis belt in sub-Saharan Africa(14)(15)(16)**.** The overall incidence of meningococcal disease in Europe and North America is 1–3/100 000 of population, but incidence rates may reached 1000/100 000 or 1% of the population during severe epidemics in sub-Saharan Africa(11)(17)**.**

Asymptomatic pharyngeal carrier of *N. meningitidis* account for 5-10% of the normal general population(18). There are several risk factors associated with asymptomatic meningococcal carriage that increase meningococcal carriage dramatically. These include crowded and closed or semi-closed settings populations (e.g. military recruit camps, dormitories or residential schools, Hajj and Umrah pilgrims) transmission increases dramatically and carriage can reach levels of 40%-80% where persons live, work, and socialize together(7)(9).Other risk factors that increase risk of being a meningococcal carrier are respiratory tract infections of viral or bacterial origin , personal behavior's (e.g. coughing, kissing, active as well as passive smoking) and low socio-economic status(4)(7).

Meningococcal carriage prevalence differs within and between countries, varying across age groups and over time. Rate is also influenced by contact with cases and the epidemic/endemic situation(7). Point-prevalence carriage rates in Europe and the United States have been estimated to range from 10 to 35% in young adults and it is likely that, at one time or another during life, most individuals are colonized with meningococcal(4)**.** In comparison ,African Meningitis Belt, which spans across more than 20 African countries from Senegal to Ethiopia, report on carriage rates of up to 35%(19)**.** During epidemics,second half of the dry season in cycles of 2–12 years,

African meningitis belt carriage prevalence of the outbreak strain can increase by a factor of 10 or higher and seasonal hyperendemicity is observed every dry season between January and May (20)(21)(22). Age-related carriage rates up to 20-50% being reported in late adolescence and carriage rates are very low in the first years of life, sharply increase in teenagers and carriage rates in older ages are lower than 10%(19)(23).

In addition, meningococcal serogroup distribution is highly regional and cyclical in the world (24).In Americas, incidence of disease is caused by serogroups C and B, although serogroup Y in some countries and W-135 is becoming increasingly problematic as well. In European countries, incidence of disease caused by serogroup B strains, particularly in countries that have introduced serogroup C meningococcal conjugate vaccines. Incidence of disease by serogroup B also predominates in Australia and New Zealand, whereas in Australia because of the control of serogroup C disease through vaccination. Incidence of diseases caused by serogroup A and C strains predominates in Asia (11). Outbreaks in sub-Saharan Africa were caused by serogroup C, W135 and X of *N. meningitidis* but most of epidemics were due to serogroup A until the introduction of a meningococcal serogroup A conjugate vaccine (MenAfriVac) in 2010(20)(21)**.** In Ethiopia, isolates of serogroup A,B, C, W, X and Y were also identified recently(19)(5).

The continuing increase in antibiotic resistance in many bacterial pathogens is a serious public health threat worldwide *Neisseria meningitidis*, causing meningitis and septicemia has been an exception, in that it has generally remained susceptible to the antibiotics used for treatment and prophylaxis. Disseminated disease requires prompt treatment with an extended-spectrum cephalosporin or chloramphenicol. (13).Over the past decade, decreased susceptibility to penicillin has been reported from a large number of countries in all continents. A few beta-lactamase-producing strains have also been described in South Africa, Spain and Canada(25). Intermediate susceptibility/resistance to ciprofloxacin and resistance to rifampicin have also been reported from several countries(13). Furthermore, although rare, there have been reports of chloramphenicol-resistant *N. meningitidis* isolates from Australia, France and Vietnam(13). Recent study in Ethiopian reported resistance for Ceftriaxone (69.4%), sensitive to Ciprofloxacin(83.7%) and Penicillin sensitivity (4.2%) intermediate resistance (83.3%) and complete resistance (12.2%) of all isolates(19)**.**

Implementation of vaccination in lowering carriage rate of meningococci is the most effective measures for preventing IMD; because carriage is a necessary precursor to IMD (25). Asymptomatic carriers are the major source of infection so that, Immunoprophylaxis is one of the most effective methods of inducing herd protection by preventing pharyngeal meningococcal acquisition(18).Conjugated vaccines that provide direct protection against 5 of the 6 major disease causing meningococcal serogroups (A, B, C, W, and Y) are currently available. In African meningitis belt following implementation of MenAfriVac ,serogroup A vaccine in 2010 led to a decrease of serogroup A carriage in all age groups and incidence had fallen 10-fold by 2013 (26). Similarly, in European countries introducing meningococcal conjugated vaccine C reduced carriage of serogroup C among population(25).

In Ethiopia, limited studies identified among high-risk populations such as military recruits, prisoners, and university students, students living in halls of residence, school children, and close contacts of disease cases(19). On the other hand, data regarding pharyngeal meningococcal carriage, serotypes involved and susceptibility testing for antibiotics conventionally used for treatment and prophylaxis of invasive meningococcal diseases are few in this country(27).

The aim of this study is to determine the characteristics of pharyngeal *Neisseria meningitides* carriage in one of high risk group among prisoners as well as susceptibility to antimicrobial commonly used for treatment and prophylaxis among asymptomatic carriers of Ethiopian prisoners in Jimma Town.

1.3 Significance of the Study

Pharyngeal asymptomatic meningococcal carriage is common in any health population especially in African meningitis belt including Ethiopian in region where periodical outbreak occurs in dry season. Currently there is limited data on meningococcal carriage rate in closed and crowded population such as among prisoners.

Current study among prisoners is essential to understand the rate of meningococcal carriage and antibiotic susceptibility pattern. Furthermore, the study on meningococcal carriage will avail up-to-date information regarding serogroup, rate of *N. meningitidis* carriage and antibiotic susceptibility among high risk group in Ethiopia.

2 Literature Review

2.1 Meningococcal Carriage Rate

Humans are the only natural hosts for meningococcus, and carriage in the nasopharynx, however, carriage is both a prerequisite of invasive disease and essential for transmission(3). According to different studies the rate of meningococcal carriage varies among different group. Historically, semi-closed populations have had high meningococcal carriage rates and have experienced recurrent outbreaks like university students, and military barracks(20)**.** The studies revealed that meningococcal carriage in high risk group such as, university student and military barracks was higher than normal general population(21). In African meningitis belt wide range of meningococcal carriage prevalence was also documented(20)**.**

The studies in European countries reported different carriage rate in different setting. Study conducted in 2014, among undergraduate students at the university of Coimbra, Portugal, revealed that pharyngeal meningococcal carriage rate was 13.3% (28). Study conducted in 2016 at university of Nottingham in United Kingdom, revealed that *N. meningitidis* carriage rate in first-year university students increased from 14% in late September,2015 to 39% by mid-November, 2015 and reached 46% in March,2016 (2). Studies conducted in Poland among professional soldiers were found to be 65 (5.2 %) carriers of *N. meningitidis*(29). Study conducted in 2011 among Turkish recruits upon entry to the military service revealed that meningococcal carriage rate was 4.2% (30). A cross sectional survey conducted between January and March 2013 in a military unit in Poland found that meningococcal carriage was (9.6%) among unvaccinated soldiers(18).

The studies conducted in Asian countries reported different carriage rate among military service and among students .Cross sectional study from Korea in 2012 among university freshmen students showed that carriage rate was 11.8% (16/136) at the first culture and 4 weeks later, carriage rate was 14.1% (18/128) at the second culture and over all carriage rate was 12.9%(34/264) (31). A cross-sectional study conducted in north Indian in 2014 among college freshmen student identified 1.5% of N. meningitis carriage rate (32). In 2016, a cross-sectional study conducted in Iran among military service before vaccination *N. meningitidis* carriage rate were identified in 8% of individuals. (33). Study conducted in Malaysian in 2007 among

army recruits in a training camp revealed that point prevalence of *N. meningitidis* carriage was 37.0% (34).

Study conducted in United State of America from 2015-2016 among university students during first cross-sectional study 167 (14%) individuals were identified as carrier of *N. meningitidis.* The second cross-sectional study identified 183 (17%), third cross-sectional study identified 110 (11%), and fourth cross-sectional study identified 163 (17%). The overall carriage rate was identified 622 (15%) individuals (35).

The studies conducted in Latin American countries report different carriage rate among military service and among students. Cross-sectional study in subjects aged 1-24 years in the city of Embu das Artes Sao Paulo, Brazil, in 2012 showed that pharyngeal carriage prevalence of *Neisseria meningitidis* was 9% (36). Cross-sectional study conducted among university students in Santiago, Chile in 2012 revealed that (4%) of *N. meningitidis* carriage rate (37).

Cross-sectional study, population-based survey conducted among general population in Mali in May, 2010 showed that 5% of individuals identified as *N. meningitidis* carriers. During the longitudinal follow-up study starting from July, 2010 found that the mean duration of carriage was estimated to be 2.9 months(38).

The studies conducted in Ethiopia reported different carriage rate in different setting **.**A cross-sectional study at Arba Minch, southern Ethiopia in 2014 revealed that carriage prevalence was 6.6 % (5). Cross-sectional carriage study conducted in 2017 at Addis Ababa Ethiopia among apparently healthy school children and adolescents revealed that *Neisseria meningitidis* carriage was (20.4%) (19)**.**

2.2 Serogroup Distribution

Studies conducted in Europeans countries reported different types of serogroup distribution .A cross sectional survey conducted between January and March 2013 in a military unit in Poland found most frequently identified serogroups among the carriers serving in the military facility were serogroup B (28 %), followed by Y (25 %) and C (22 %) (18). Study conducted in 2014, among undergraduate students at the university of Coimbra, Portugal, identified serogroups (A-0%, B-5.3%, C-0.3%, W-0.2%, X-0.2% and Y-1.7%) and the rest isolates were NG (28). Studies conducted in Poland among professional soldiers identified serogroups B 25(38.5 %), Y 4(6.15 %), E29 2(3.1 %), C 2(3.1 %),W 2(3.1 %), and A 1(1.5 %) (29). Study conducted in 2016

in United Kingdom, identified predominant *N. meningitidis* serogrouping W (62%) among university students (2). Study conducted in 2011 among Turkish recruits upon entry to the military service identified serogroups Y(15.6%), W135 (10.8%), C(9.6%), B(6.1%), A(2.4%), and (55.4%) detected as nongroupable (30).

Studies conducted in Asian countries also reported different types of serogroup distribution. Cross sectional study conducted in 2012 in Korea among university freshmen students found serogroup C was the most frequent serogroup that were 5 isolates, while 3 isolates were from serogroup B, 8 isolates were NG. The second culture identified additional serogroup 29E and serogroup W135(31). In 2016, a cross-sectional study conducted in Iran among military service before vaccination isolated serogroups C, A, Y, W-135, and X with frequencies of 50%, 22.2%, 16.6%, 5.5%, and 5.5%, respectively (33). Study conducted in Malaysian in 2007 among army recruits in a training camp isolates W135(4.8%), A(3.33%) and 81.4% belonged to serogroup X, Y or Z(34).

Studies conducted in America and Latin American countries reported different types of serogroup distribution. Study conducted in United State of America from 2015-2016 among university students identified serogroup B, W, X, Y and NG (35). Cross-sectional study conducted among university students in Santiago, Chile in 2012 identified serogroup B, W and nongroupable (37). Cross-sectional study in subjects aged 1-24 years in the city of Embu das Artes Sao Paulo, Brazil, in 2012 identified predominant serogroup C (18.4%) followed by B (12.6%) , NG (60.9%) and the rests were Y, W, X, and E (36).

Cross-sectional study, population-based survey conducted in general population in Mali in, 2010. This study identified six isolates of serogroup W and nine isolates of serogroup Y and majority of isolates were classified as nongroupable. No A, C and X carriers were identified (38).

Studies conducted in Ethiopia reported different types of serogroup distribution. Studies conducted in Gondar, Hawassa and Addis Ababa among patients with suspected bacterial meningitis in Ethiopia, 2012–2013. The serogroups detected in this study were A (11), W (7), C (1), X (1) and NG (7) or (not serogrouped as A, C, Y, W, or X) (39). Cross-sectional carriage study conducted in 2017 at Addis Ababa Ethiopia among apparently healthy school children and adolescents revealed that the rate of sero-groups were ,W135 (42.6%), C (23.4%), A(12.2%) while the rest meningococci were serotypes B,X, Y and non-serogroupable variants(19)**.** A cross-sectional study at Arba Minch, southern Ethiopia in 2014 revealed that non-groupable isolates

were dominant (76.4 %) followed by serogroups X (14.0 %), W (5.9 %) and the rest isolates were Y, C and B while serogroup A was not found (5). Study conducted in Gurage Zone, Southern Ethiopia identified isolates of serogroup X, Y, W135, and non-determinant (ND)(27). Study conducted in Jimma southwest Ethiopia from March 2013 to December 2015 identified *Neisseria meningitidis* serogroup ACYW (40).

2.3 Antibiotic Susceptibility

Antimicrobial resistance of bacteria is a worldwide problem(41).Study conducted in Swedish from 1995-2008 to determined susceptibility of *Neisseria meningitidis* isolates to 7 antibiotics reveal that ciprofloxacin appropriate for prophylaxis. All isolates (100%) were *b*-lactamase-negative and highly susceptible to cefotaxime and ciprofloxacin. Mainly all isolates (99.9%) were susceptible to rifampicin and chloramphenicol, two percent of the isolates were resistant to penicillin V and 50% of these were serogroup B isolates (13).

Study conducted in Malaysian in 2007 among army recruits in a training camp revealed that antibiotic susceptibility patterns of *N. meningitidis* isolates were susceptibility to chloramphenicol, rifampicin, cefotaxime and levofloxacin and 85% of the strains were resistant to cotrimoxazole and 12.5% resistant to penicillin(34).

Study conducted between 2000 and 2006 from 18 African countries mainly those within the meningitis belt towards susceptibilities of *N. meningitidis* isolates to 11 antibiotics revealed that all isolates were susceptible to ceftriaxone, chloramphenicol, and ciprofloxacin. No isolate produced lactamase, 52% of the isolates were resistant to tetracycline, 74% were resistant to erythromycin, and 94% were resistant to sulfadiazine. Three of the isolates (2%) displayed reduced susceptibility to penicillin G and two of these isolates were in serogroup A (isolated in Ethiopia and Somalia), and one was in serogroup Y (isolated in Senegal) (12).

Cross-sectional carriage study conducted in 2017 at Addis Ababa Ethiopia among apparently healthy school children and adolescents showed resistance for ceftriaxone were (69.4%), sensitive to ciprofloxacin were (83.7%) , sensitive to penicillin were (4.2%) while, the bulk of meningococci isolates were intermediate resistance to Penicillin (83.3%) and Penicillin complete resistance isolates were (12.2%) (19)**.**

Study conducted among asymptomatic carriers in Gurage Zone, Southern Ethiopia showed that isolates of *Neisseria meningitidis* resistant were (62%) to Cotrimoxazol, (60%) to Ciprofloxacin,

(14%) to Cefotaxime, (13%) to Ceftriaxone, (11%) to Meropenenem, (8%) to Minocycline, (7%) to Rifampin, (5%) to Azithromycin, (4%) to Chloramphenicol and (3%) to Levofloxacin. Cotrimoxazol resistant were the highest (62%) and then Ciprofloxacin resistant were (60%) of isolates (27). Study conducted in Gondar, northwest Ethiopia between September 2002 and August 2003 showed that N.meningitidis was found to be resistant to co-trimoxazole 5(50%), chloramphenicol 3(30%), gentamicin 3(30%) and ampicillin 2(20%)(39). Study conducted in Jimma, southwest Ethiopia from March 2013 to December 2015 showed that isolates were susceptible to penicillin and small inhibition zone around the ceftriaxone disc (40).

2.4 Risk Factor for Carriage

There are several risk factors associated with asymptomatic meningococcal carriage that increase meningococcal carriage dramatically (7) . Cross sectional study among British teenagers showed that *N. meningitidis* carriage rate was positively associated with cigarette smoking, intimate kissing, and pub or club patronage. While, recent antimicrobial drug use was negatively associated with meningococcal carriage rate. The age group from 16 years to 18 or 19 years was attributable to the rise in meningococcal carriage rate (3). Study conducted in United State of America from 2015-2016 among university students found that male sex, being 20 years of age, smoking, and attending social mixing events ≥1 time per week were associated with increased carriage of *N. meningitidis*. While, recent antibiotic use was associated with decreased carriage of *N. meningitidis* (35). Studies conducted in Poland among professional soldiers found active smoking associated with increasing of meningococcal carriage rate in military(29). Cross sectional study conducted in 2012 in Korea among university freshmen students showed that visiting to pubs was associated with carriage of *N. meningitis*. Respiratory tract infections and active smoking were high risk to become meningococcal carriage in Korea (31).Cross-sectional study in subjects aged 1-24 years in the city of Embu das Artes Sao Paulo, Brazil, in 2012 showed that risk of pharyngeal carriage increased with the number of household members(36).

CONCEPTUAL FRAME WORK

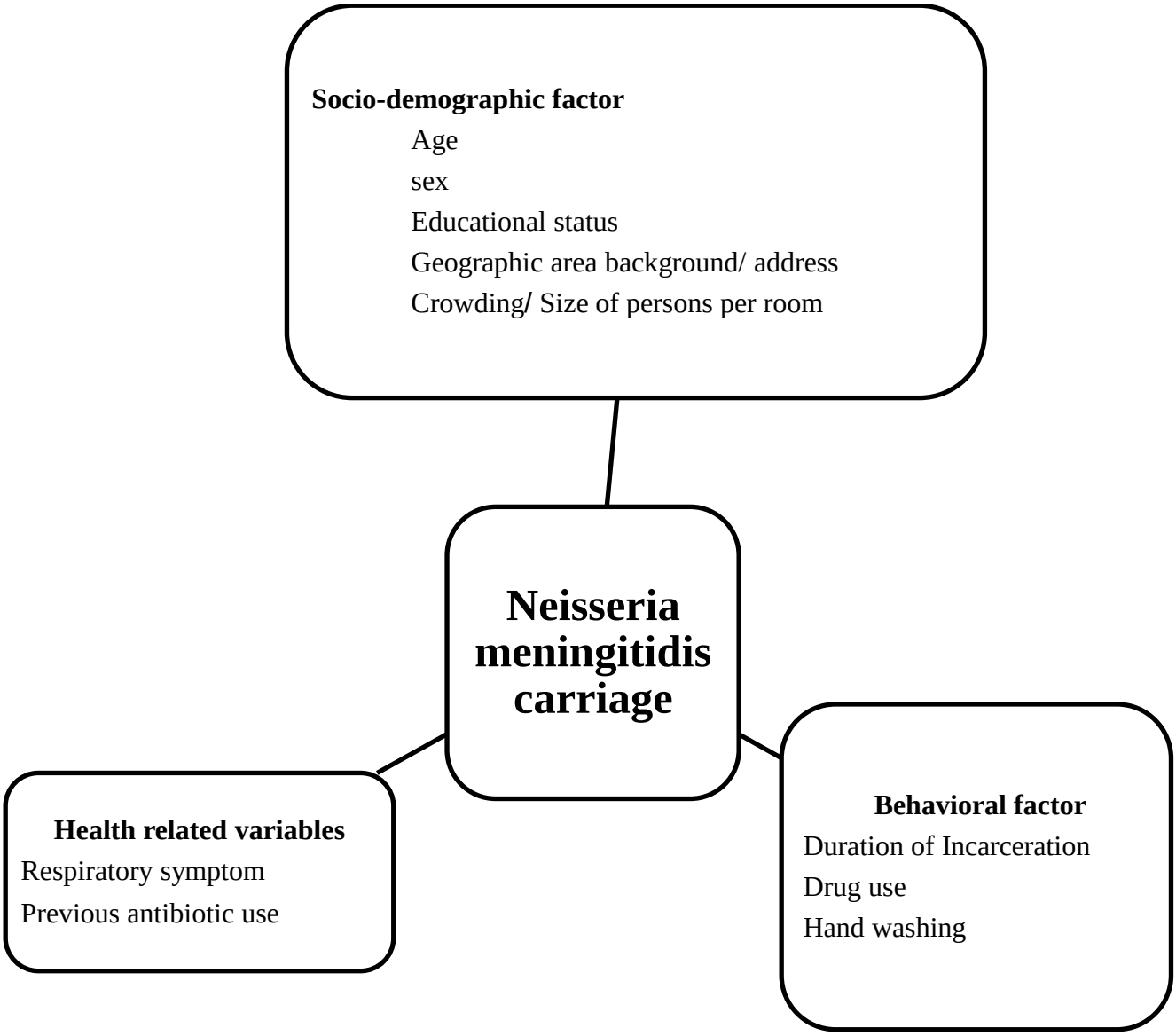

Figure 1: Conceptual Frame Work for N. meningitidis carriage factor relationship, Jimma Ethiopia, 2019

3 Objectives

3.1 General Objective

Determine N*eisseria meningitidis* carriage rate and its antibiotic susceptibilities among prisoners at Jimma Zonal Prison, southwest Ethiopia, May-October, 2019.

3.1.1 Specific Objectives

- To determine the magnitude of N*eisseria meningitidis* carriage among prisoners at Jimma zonal prison.
- To determine N*eisseria meningitidis* isolates serogroup among prisoners at Jimma zonal prison.
- To determine antibiotic susceptibility pattern of N*eisseria meningitidis* isolates at Jimma zonal prison.
- To assess factors associated with N*eisseria meningitidis* carriage rate in prisoners at Jimma zonal prison.

4 Material and Method

4.1 Study area and Period

The study was conducted from May to October, 2019 in Jimma Zonal Prison located in the Jimma town. Jimma town is the largest town situated in Jimma zone, Oromia region, in the Southwest Ethiopia. It is far from Addis Ababa, Ethiopia with driving distance of 346km and straight line or air distance 256km.The rainy season lasts 7 months from March 20 to October 21, with a greater humidity of 86%. The drier season lasts 5 month from october 21 to march 20 and December is lowest humid month with humidity of 8%.

Oromia regional state have 17 Zonal prisons administrative commision which are the largest prisons in Ethiopian context(42). Jimma Zonal administrative prisons is one of zonal prison in Oromia regional state. Jimma Zonal administrative prison had 2164 total prisoners in 19 different size rooms of these 2054 were male prisoners and 110 were female prisoners. The size of rooms ranging from smallest room size of 30m2 (323ft2) to largest room size of 195m2 (2098ft2) while the average room size is 141m2(1517ft2).

The rooms of prisoners classified in to two groups based on sex as male rooms and female rooms. While male had also category of juvenile prisoners from age of 9-17 years old and adult prisoners from age of 18 and above years old. Female prisoners live in one small and one medium size rooms each contain 34 and 76 individuals respectively. The male juvenile prisoners live in three small equal size rooms each contain 33,34 and 30 individuals respectively from room 1-3. Majority of adult prisoners live in three large and nine larger size rooms. The three large size rooms contain 120,118 and 122 individuals respectively from room 1-3. Nine larger size rooms each contain 176, 177, 166, 162, 160, 171, 165, 157 and 165 individuals respectively from room 1-9. While, the rest adult male prisoners live in one smallest and one medium size rooms each contain 12 and 89 individuals respectively (*Jimma Zonal Prison daily record and attendance log book, 2019*).

4.2 Study Design

A cross-sectional study was conducted on meningococcal carriage among volunteer prisoners enrolled in the study after offering them.

4.3 Participants

All prisoners in Jimma Zonal Prison were the source population.

4.4 Study participants

All sampled prisoners in Jimma Zonal Prison

4.5 Inclusion criteria

Participants those jailed for at least 7 days and those who gave consent were included in the study.

4.6 Exclusion criteria

Participants those were HIV patient and patient on immune-suppressing therapy were excluded. Those participates soon to be released from prison were also excluded.

4.7 Sample Size

Stratified random sampling technique was employed by forming two strata. (1) Strata of male consist of 2054 males dwelled in 17 different size rooms and (2) strata of female consist of 110 females dwelled in 2 different size rooms. Then sample size was determined for each stratum and for each room by proportional allocation. Final simple random sampling was employed by lottery method to recruit from prepared list of sample frame in each stratum and room. All prisoners were offered voluntary to participation in the study and in keeping with the inclusion and exclusion criteria were recruited till the sample size were attained. Using single proportional formula sample size of 275 participant calculated citing the prevalence noted by *Alemayehu T, et al, 2017* from Addis Ababa Ethiopia: p = 0.204, z = 1.96 d=0.05(19).

Single proportional formula $\boldsymbol{n = \frac{z^2 a/2p(1-p)x^2}{d^2} + 10\%}$

- p = 0.204
- z = 1.96
- d=0.05

n= 1.962 x0.205x0.795/0.052 =250+25

***n*= <u>275</u>**

- n= sample size
- d=Margin of error
- p= proportion
- Confidence and significance levels=95% and 5% respectively.
- By adding 10% non-response rate, final sample size=**<u>275</u>**

Proportional allocation formula $\boldsymbol{n = \frac{n}{N} * N1 + \frac{n}{N} * N2}$

Male =263

Female =12

4.8 Variable

4.8.1 Dependent variables

Neisseria meningitidis carriage

4.8.2 Independent variables

Socio-demographic factor

- Age
- Sex
- Educational status
- Geographic area background/ address
- Crowding/ Size of persons per room

Behavioral factor

- Drug use
- Hand washing
- Duration of Incarceration

Health related variables

- Respiratory Symptom
- Previous antibiotic use

4.9 Operational Definition

Antibiotic Use:-The participants who were took antibiotics for fever and diarrhea, for various types of respiratory symptom such as coughs and colds during the 3 months before 14 days prior to pharyngeal swab.

Respiratory symptom: Participants who show common symptoms for upper and lower respiratory tract include the cold, laryngitis, pharyngitis/tonsillitis, acute rhinitis, and acute rhino sinusitis and pneumonia (CDC, WHO).

Drug use:-Participants who did not practice smoking, taking alcohol, chewing khat and were not passive smoker in last three month.

Meningococcal :- *Neisseria meningitidis*,a frequent cause of meningitis (43).

Carriage:– Asymptomatic commensal of *Neisseria meningitidis* of humans, which normally colonizes the mucosa of the upper respiratory tract without causing invasive disease, a phenomenon known as carriage (4).

High-Risk Groups :-Historically, closed populations have had high rates of meningococcal carriage and have experienced recurrent outbreaks ,are recommended for targeted vaccination in many countries(20).

Prisoner: -Any individual involuntarily confined or detained in a penal institution. Individuals incarceration in a penal institution, and individuals detained pending arraignment, trial, or sentencing(44).

Overcrowdings:- Considered as standard of more than 2 person per room of 110 ft2 (10.2m2) if room is avialble as sleeping accommodation of a type normally used either as a bedroom or as a living room (45). Cell used for prisoner accommodation should measure at least 2m between the walls of the cell and 2.5m between the floor and the ceiling. Minimum standard 2 prisoners: at least 10m² of living space + sanitary room 1m² to 2m² and maximum 4 prisoners: at least 18 m² of living space + sanitary rom 2m²; sufficient ventilation to ensure a constant renewal of the air inside the cells (46).

Passive smoking: - Is the inhalation of smoke, called second hand smoke (SHS), or environmental tobacco smoke (ETS), by persons other than the intended "active" smoker. It occurs when tobacco smoke enters an environment, causing its inhalation by people within that environment(47) and the practice of this must be within last three month by participant.

Active Smoking: - Inhalation tobacco smoke directly by participant currently or recently within last three months once per a day.

4.10 Data collection methods

Study participants were recruited after a written informed consent was obtained from participant. Socio-demographic data and information concerning factors associated for meningococcal carriage were collected using a structure questionnaire.

4.11 Specimen Collection and Microbiologic Procedures

I. Specimen Collection and Processing

After a written informed consent specimens were collected from recruited participants from posterior wall of the pharynx. Swabs were collected by a trained medical microbiologist using pre-moistened sterile cotton swabs through the open mouth, following standard operating procedure by touching the tip of a swab against pharyngeal and tonsillar fossa and then passing it in an upward semi-circular motion over the soft palate to the opposite side. After collection, samples were inserted immediately into Amie charcoal transport medium and were transported to the laboratory within one hour in cold box about 2-8^{o}C (6) (48).

II. Laboratory Assay for Isolation and Identification

Isolation and identification was undertaken in the microbiology laboratories of Jimma University, Jimma Ethiopia. Pharyngeal swabs were plated onto selective Modified Thayer-Martin (MTM) agar by rolling and streaking of swab over plates using a sterile loop. Selective Modified Thayer-Martin (MTM) was supplemented with Vancomycin, Colistin, Nystatin and Trimethoprim (VCNT) inhibitor. Inoculated plates were incubated at 35-37˚C under a 5% - 10%CO_2 enriched atmosphere for 72 hours. Plates were examined for growth of typical colonies every 24 hours. The colonies suggestive of *N. meningitidis* on the plates tested for gram staining for those giving gram-negative diplococci results subjected to oxidase activity for giving oxidase positive. All oxidase positive, Gram-negative diplococci were sub-cultured on chocolate agar before performing biochemical identification tests in order to ensure that adequate viability and undergoing biochemical carbohydrate utilization tests (glucose, maltose, lactose and sucrose) on Cystine Trypticase agar (CTA) used for further confirm colonies. Gram negative, oxidase positive, glucose positive, maltose positive, lactose negative and sucrose negative diplococci was confirmed as *Neisseria meningitides.* Those identified as *N .meningitidis* on these tests were

undergoing slide-agglutination serogrouping method employed using mixtures of colonies with standard antisera for *Neisseria meningitidis* serogroups A, B, C and W/Y those identify a prioritized as the likely most prevalent serogroups. The presence of agglutination during mixing used to check for the specific serotypes. If negative and agglutinate for all serogroup A, B, C and Y/W, it was classified as non-serogroupable (NG) (6).

III. Antibiotic Susceptibility Testing

Susceptibility testing for antibiotics used for treatment of patients and for prophylaxis (prophylactic treatment) was done using disc diffusion method. A standardized 0.5 McFarland used to confirm inoculum density. Colonies were inoculated on Muller Hinton chocolate agars prepared from sheep blood and inoculated at 37˚C under a 5%-10% CO2 enriched atmosphere for 24 hours thereafter at 24-hour intervals until 96 hours. Susceptibility to antibiotics were confirmed according to the Clinical and Laboratory Standard Institute (CLSI-2017) of Anti-microbial chemotherapy guidelines for penicillin, ceftriaxone ,chloramphenicol, ciprofloxacin and rifampicin *Table 1* and determined for all isolates, using (Liofilchem, Oxoid) Anti-microbial product(49).

Table 1 : Breakpoints for antimicrobial agent (CLSI-2017)

Antimicrobial Agent	Disk Content	Interpretive Categories and Zone Diameter Breakpoints (nearest whole mm)			Interpretive Categories and MIC Breakpoints (µg/mL)			Comments
		S	I	R	S	I	R	
Penicillin	-	-	-	-	≤0.06	0.12-0.25	≥0.5	
ceftriaxone	30ug	≥34	-	-	≤0.12	-	-	
Ciprofloxacin	5ug	≥35	33-34	≤32	≤0.03	0.06	≥0.12	prophylaxis
Chloramphenicol	30ug	≥25	20-25	≤19	≤2	4	≥8	
Rifampin	5ug	≥25	20-24	≤19	≤0.5	1	≥2	prophylaxis

S- Sensitive, I- Intermediate, R-Resistance

4.12 Quality Control

Questionnaire was translated to local languages (Amharic and Afaan Oromo) and retranslated by other translators to English. Training was given for the data collectors and the data collected were checked for the completeness by principal investigators and double enterey check in Epidata.

All prepared media were tested for growth support, production of proper biochemical reactions and susceptibility using ATCC quality strain of *(Neisseria meningitidis* serogroup A (ATCC). uninoculated plates were check for sterility by incubating at appropriate environment and temperature. Serogrouping of isolates were done after mixing colonies with normal saline to check auto agglutination. Standard antisera also mixing with prepared by manufacturer positive polyvalent control latex and latex negative controls separately on a slide to confirm purity by observing for precipitates. Finally standard antisera test for serogroup A (ATCC) strain (PASTOREXTM MENINGITIS) (49)(6).

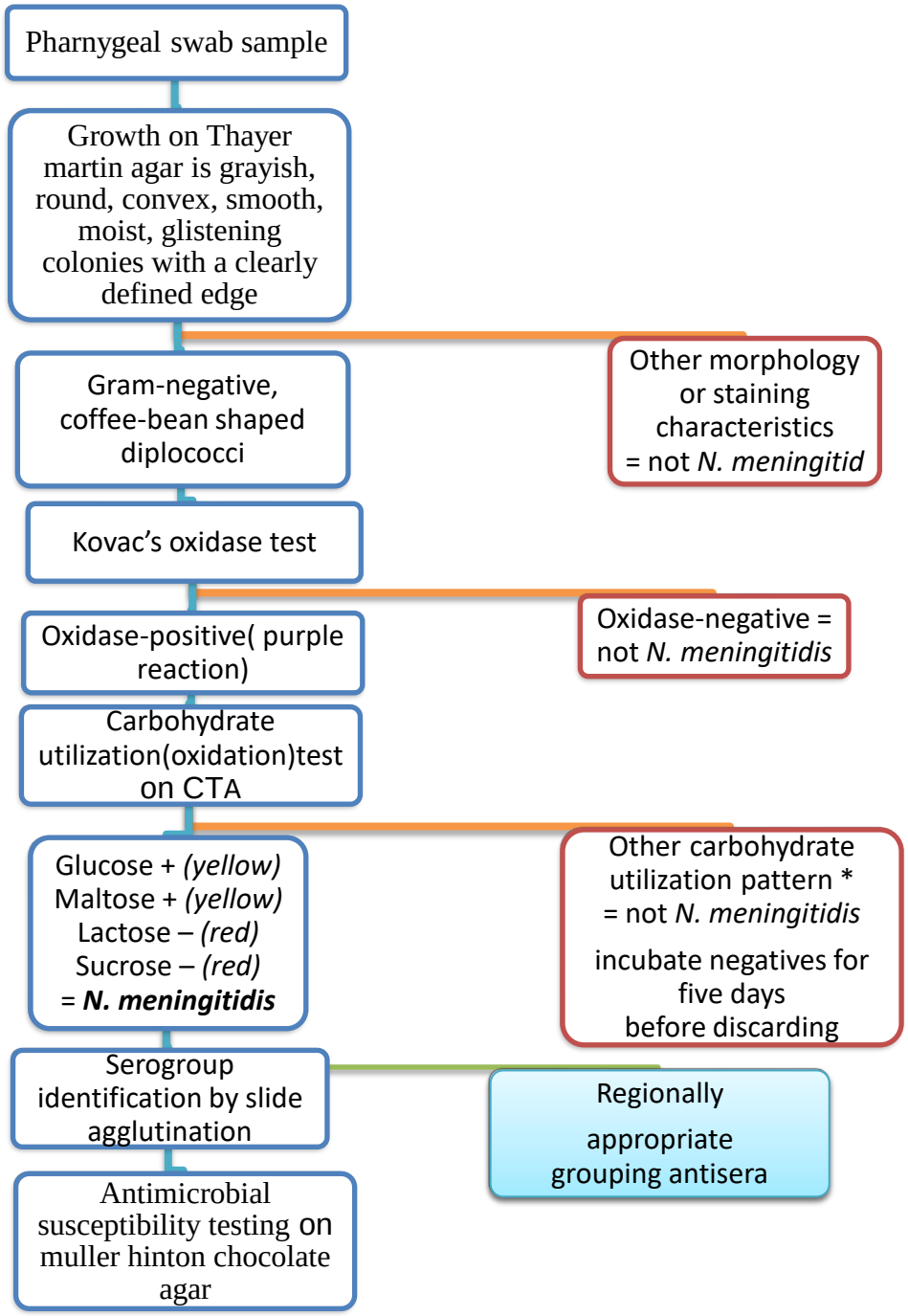

Figure 2: Microbiological Standard Procedures flow chart, Jimma Ethiopia, 2019.

4.13 Statistical analysis

All data were recorded on data collection forms and double-entered in to Epidata. All recorded data were transferred to SPSS version 21. Data were summarized using descriptive statistics of bar graphs, and tables. Binary logistic regression analysis was conducted to analysis the association between factors and menningococcal carriage of 95% confidence interval. The p.value less than 0.05 determined significant association between factors and menningococcal carriage rate. Pharyngeal meningococcal carriage rate was characteristics in proportion.

4.14 Ethical considerations

The study protocol was approved by the department of Medical Microbiology, college of Health Sciences of Jimma University. Ethical clearance was obtained from Jimma University IRB of Institute of Health. The permission to undergo the study was also granted by Jimma Zonal Prison Administration Commission and written informed consents were taken from all study participant.

4.15 Dissemination of the Results

The research finding was disseminated to Jimma University graduate research program, School of Medical Laboratory Sciences. Furthermore, it was disseminated to Jimma Zonal Prison Administration commission, Jimma zone health bureau. Finally, manuscript was prepared and submitted to national or international peer reviewed journal for publication.

5 Results

5.1 Socio-Demographic, Behavioural and Health Related Factor

A total of 275 participants were included in the study (263 males and 12 females).The mean age of participant was 27.65 years with minimum age of 16 years and maximum age of 77 years. Crowded living conditions were seen in all rooms with average of one individual per 1.34m^2 (14.45ft^2) area (standard minimum of 1 person per 5.11m^2 (55$ft^{2)}$. The median of participants spent in the prison was (14) month with minimum (1) month and maximum (208) months **Table 2**. Current or recent active cigarette smoker participants within last three month were 18.2 %; whereas around 28.7% of participants were current or recent passive smoker within last three month; 15.3% of participants used to chew khat in last three months and 43.6% of participants were no use drug in last three month **Table 2.** The participants those got respiratory symptom in last three month were 39.6 % and participants history of taking antibiotics for fever and diarrhea for various types of respiratory symptom such as coughs and colds during the 3 months to pharyngeal swab were (42.9%) **Table 2**.

Table 2: Characteristic of socio-demographic, behavioural and health related factor of study Participants, Jimma Ethiopia, 2019.

Factors		Non-carrier	Carrier
		n= 237 (86.2%)	**n = 38 (13.8%)**
Age category	16-20 years	71 (30.0%)	22 (57.9%)
	21-25 years	60 (25.3%)	11(28.9%)
	26-30 years	45 (19.0%)	2 (5.3%)
	Older than30 years	61(25.7%)	3 (7.9%)
sex	Male	226(85.9%)	37(97.4%)
	Female	11(14.1%)	1(8.3%)
Residential background	Rural	130(54.9%)	23(60.5%)
	Urban	107(45.1%)	15(39.5%)
Active smoking	Yes	23(53.5%)	20(46.5%)
	No	214(92.2%)	18 (7.8%)
Passive smoking	Yes	68 (86.1%)	11 (13.9%)
	No	169 (86.2%)	27(13.8%)
Chewing Khat	Yes	34(81.0%)	8 (19.0%)
	No	203 (87.1%)	30 (12.9%)
Non drug user	Yes	114 (95.0%)	6 (5.0%)
	No	123 (79.4%)	32 (20.6%)

Duration of stay in prison	Median(mode)	14(3)	
	Min & max	1 & 208	
Incarceration history	Yes	11 (78.6%)	3 (21.4%)
	No	226 (86.6%)	35 (13.4%)
Incarceration frequency	One	10 (83.3%)	2 (16.7%)
	Two	1 (50.0%)	1 (50.0%)
Educational status	No education	66 (27.8%)	9 (23.7%)
	Primary	114 (48.1%)	18 (47.4%)
	Secondary	49 (20.7%)	10 (26.3%)
	Diploma and above	8 (3.4%)	1 (26.3%)
Overcrowded	Yes	237	38
	No	0	0
Hand washing habit	With water alone	176(85.0%)	31(15.0%)
	With soap and water	61(89.7%)	7(10.3%)
Respiratory symptom	Yes	87 (79.8%)	22 (20.2%)
	No	150 (90.4%)	16 (9.6%)
Antibiotic use	Yes	108 (91.5%)	10 (8.5%)
	No	129 (82.2%)	28 (17.8%)

5.2 Isolates of *Neisseria meningitidis* and Serogroup Characteristic

Out of 275 study participants, 38(13.8%) participants were found to harbour *N. meningitidis* **Table 2.** Serogroups isolated from participants were serogroup A 2(5.3%), serogroup B 5(13.2%), serogroup C 7(18.4%), serogroup W/Y 11(28.9%) and Non-groupable variant 13(34.2%) **Figure 3**.

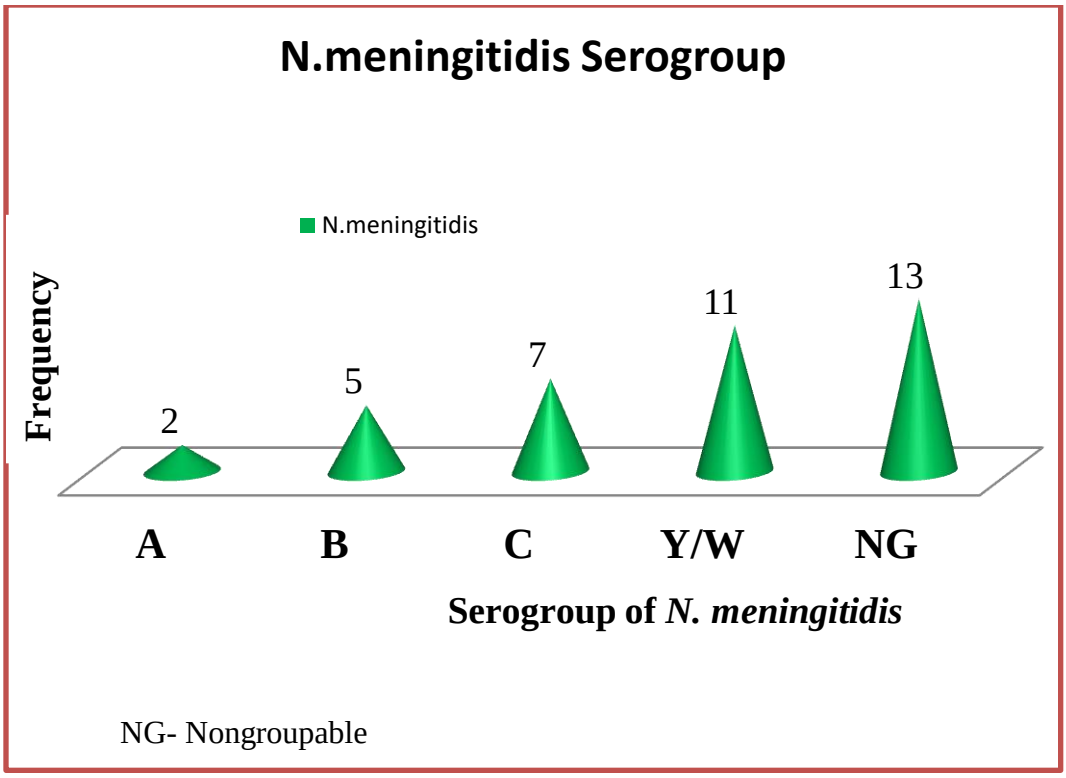

Figure 3: Characteristic of isolates serogroup, Jimma Ethiopia, 2019.

5.3 Serogroup Distribution of *Neisseria meningitidis* Isolates by Age Group

Higher percentages of positive subjects per age group were observed in the age categories of 16-20 (57.9%) and 21-25 (28.9%) years respectively. Serogroup B was more dominant among age group of 16-20 years **Table 3**.

Table 3: Distribution of isolates of *Neisseria meningitidis* serogroup by age group, Jimma Ethiopia, 2019.

	Age group				Total
	16-20 years	21-25 years	26-30 years	Above 30 years	
A	1(2.6%)	0	0	1(2.6%)	2(5.3%)
B	4(10.5%)	0	0	1(2.6%)	5(13.2%)
C	5(13.2%)	2(5.3%)	0	0	7(18.4%)
W/y	6(15.8%)	4(10.5%)	1(2.6%)	0	11(28.9%)
NG	6(15.8%)	5(13.2%)	1(2.6%)	1(2.6%)	13(34.2%)
Total	22(57.9%)	11(28.9%)	2(5.3%)	3(7.9%)	38(100.0%)

5.4 Antimicrobial Susceptibility Pattern of *Neisseria meningitidis* Isolates

Isolates of *Neisseria meningitidis* exhibited sensitive to penicillin 24(63.2%), intermediate resistance 12(31.6%) and resistance 2(5.3%); for ceftriaxone sensitive 32(84.2%) and resistance 6(15.8%); for chloramphenicol sensitive 37(97.4%) and resistance 1(2.6%); for ciprofloxacin sensitive 30(78.9%), intermediate 5(13.2%) and resistance 3(7.9%); and for rifampicin sensitive 36(94.7%) and intermediate 2(5.3%). Isolates were relatively exhibited high resistance for ceftriaxone (15.8%). Penicillin susceptibility was relatively low (63.2%), in contrast, (97.4%) of isolates were exhibited highly susceptible to chloramphenicol **Table 4**.

Table 4 Antibiotic susceptibility profile of *Neisseria meningitidis* Jimma Ethiopia, 2019

Antibiotics	Antibiotic susceptibility			Total
	Sensitive	Intermediate	Resistance	
Penicillin	24(63.2%)	12(31.6%)	2(5.3%)	38(100%)
Chloramphenicol	37(97.4%)	0	1(2.6%)	38(100%)
Ceftriaxone	32(84.2%)	-	6(15.8%)	38(100%)
Rifampicin	36(94.7%)	2(5.3%)	0	38(100%)
ciprofloxacin	30(78.9%)	5(13.2%)	3(7.9%)	38(100%)

5.5 Analysis of Factor Associated with Neisseria meningitidis Carriage Rate

Initially bivariable model of binary logistic regression was analyzed. All associated factors with $p<0.25$ were then included in a multilevel binary logistic regression model for a multivariable analysis **Table 5**.

Table 5: Bivariable binary logistic regression analysis, Jimma Ethiopia, 2019.

Variable	p.value	Crude odd ratio	95% C.I.for Crude odd ratio	
			Lower	Upper
Active Smoking	.000	10.338	4.794	22.293
Passive Smoking	.974	1.013	.476	2.155
Chewing khat	.289	1.592	.674	3.764
Non drug user	.001	.202	.082	.502
16-20 years	.004	6.300	1.798	22.076
21-25 years	.052	3.728	.990	14.030

26-30 years	.914	.904	.145	5.635
Older than 30 years	.579	1.801	.226	14.364
Residential background	.514	1.262	.627	2.539
No education	.938	1.091	.122	9.771
Primary school	.830	1.263	.149	10.709
Secondary School	.660	1.633	.183	14.548
Hand washing habit	.335	.652	.273	1.556
Duration stay in prison	.865	1.001	.988	1.014
Incarceration history	.527	.400	.023	6.848
Incarceration frequency	.318	.200	.008	4.716
Respiratory symptom	.015	2.371	1.182	4.755
Antibiotic use	.029	.427	.198	.918

Final statistical analysis of multivariable binary logistic regression model identified three factors significantly associated with increased of *N. meningitidis* pharyngeal carriage among the studied factors. These factors were being age group of 16-20 years ($p \leq 0.014$) (Odds Ratio: 5.310; 95% CI: 1.404–20.076); having respiratory symptom within three month ($p \leq 0.048$) (Odds Ratio: 2.327; 95% CI: 1.007-5.380) and current or recent within 3 month active cigarette smoking ($p \leq 0.001$) (Odds Ratio: 6.788; 95% CI 3.007-15.326) **Table 6**. While antimicrobial use within three month was negatively associated with decrease risk of *N. meningitidis* pharyngeal carriage rate ($p \leq 0.004$) (Odds Ratio: 0.263; 95% CI: 0.106–0.655) **Table 6.**

Table 6: Multivariable binary logistic regression analysis Jimma Ethiopia, 2019

Variables	p-value	Adjusted odd ratio	95% C.I for Adjusted odd ratio	
			Lower	Upper
16-20 years	.014	5.310	1.404	20.076
21-25 years	.106	3.171	.782	12.865
26-30 years	.882	.866	.130	5.754
Antibiotic use	.004	.263	.106	.655
Respiratory symptom	.048	2.327	1.007	5.380
Active smoking	.000	6.788	3.007	15.326

6 Discussion

Humans are the only natural hosts for meningococcal, and carriage is in the pharynx, however, pharyngeal carriage is both a prerequisite of invasive disease and essential for transmission (3). This study identified 13.8% pharyngeal meningococcal carriage rates among asymptomatic prisoners. This finding was higher as compared to pharyngeal carriage prevalence of 6.6% that documented among general asymptomatic population of age group from 1-29 years at Arba Minch Ethiopia in 2014(5). Comparison of previous studies other than local research among general population that reported from Mali (African meningitis belt) during the cross-sectional study pharyngeal *Neisseria meningitidis* carriers was 5.0% that was less than this study (38).And also, from Brazil, among general population from age group of 1-24 years reported 9% pharyngeal carriage prevalence of *N. meningitidis* that was also less than this study(36).

Historically, semi-closed populations had high rates of meningococcal carriage and experienced recurrent outbreaks like university students and military camp(20). However, it was less carriage rates as compared to (20.4%) *Neisseria meningitidis* nasopharyngeal carriage recorded among apparently healthy school children and adolescents in, 2017 in Addis Ababa, Ethiopia(19). Study reported among university student from United States of America from 2015-2016 over all meningococcal carriage rate showed by 15% which was higher than this study (35). However, there was outliers documented in cross-sectional study among first-year university students in United Kingdom in, 2016 meningococcal carriage among this group increased from initial 14% and reached 46% that was higher carriage rate than observed in this study (2). Similar with this study of *N. meningitidis* carriage rate was reported from Portugal among undergraduate university students in 2014 indicated by pharyngeal meningococcal carriage rate 13.3%(28).

However , among closed populations lower carriage rate than this study was reported from Koreas in 2012 among university freshmen student who were admitted to a dormitory indicated by 12.9% of *N. meningitidis* overall carriage rates (31).While, study among college freshmen in North India in 2014 and among students aged 18-24 year in Chile in 2012 found lower carriage rates of only 1.5% and 4% respectively than this study(37)(32).

Meningococcal carriage rate in closed population other that students in military service among professional soldiers serving from Poland in 2016 indicated by 5.2 % carriage rates was lower than this study(29). Study reported from Turkish, in 2011 recruits upon entry to the military

service meningococcal carriage rate was 4.2% which was lower than this study (30). Similar study from Poland in 2013 among unvaccinated soldiers meningococcal carriage rate was (9.6%) which found lower carriage rates than found in this study(18) . Other study among military service in Iran in 2016 *N. meningitidis* carriage rate was 8% which was lower carriage rates than this study (33)

In multivariable analysis being 16-20 years of age; active smoking and having respiratory symptoms in the past 3 month remained associated with increased carriage, and also, antibiotic use were remained associated with decreased *N. meningitidis* carriage rate **Table 6**. In this study being 16-20 years of age and smoking were positively associated with *N. meningitidis* carriage rate and also recently exposed to antibiotic use was negatively associated with *N. meningitidis* carriage rate that were comparable with studies documented in United Kingdom and US America (35)(3). Evidently , age groups from 15-19 years showed a significant association with increased *N. meningitidis* carriage rate documented in Brazil was comparable with this study(36).The evidence of active smoking associated with increasing of meningococcal carriage rate in Poland military also comparable with this study (29). Similar to this study, respiratory tract infections and active smoking were high risk to become meningococcal carriage in Korea (31).

N. meningitidis is divided into 13 serogroups based on the immunological specificity of the capsular polysaccharide. It has been known that pathogenic strains are encapsulated and six of these serogroups (A, B, C, W135 ,Y and X) cause more than 90% of the invasive disease worldwide(31). This study identified serogroup A 2(5.3%), serogroup B 5(13.2%), serogroup C 7(18.4%), serogroup W/Y 11(28.9%) and 13(34.2%) non-serogroupable Variant with low carriage prevalence of serogroup A observed .This finding almost identified similar serogroup distribution with serogroup documented among apparently healthy school children and adolescents in Addis Ababa Ethiopia and among general normal population at Arba Minch Ethiopia where serogroups W135, C, A, B, X, Y and NG variants were identified**.** However, at Arba Minch Ethiopia no serogroup A carriage was found. The introduction of MenAfriVac of the monovalent serogroup A conjugate vaccine is thus expected influence by lowering carriage prevalence of serogroup A (5)(19) .Study conducted in Jimma southwest Ethiopia identified *Neisseria meningitidis* serogroup ACYW which was similar to this study except serogroup B (40).

Comparing meningococcal carriage serogroup of this study to studies of other region found some similarities and variations in serogroup distribution. For example, serogroups documented in Brazil (C, B and NG) (36) ; in Chile (B, W, and NG)(37); in Koreas (C , B. NG 29E, and W13) (31) ; in United States (B,C,W,X,Y, NG)(35); in Poland (A,B ,E29, C ,Y and W)(29); in Mali (W Y and NG)(38) and in United Kingdom(B Y,C ,X and W)(23) were obvious for similarities and variations in serogroups distribution with this study.

In this study isolates of *Neisseria meningitidis* exhibited sensitive to penicillin (63.2%): exhibited sensitive to ceftriaxone (84.2%) and ciprofloxacin (78.9%). However, the isolates were highly susceptible for chloramphenicol (97.4%) and rifampicin (94.7%). Reduced to penicillin susceptibility documented in Swedish 2% isolates were resistant to penicillin (13); in Malaysian 12.5% resistant to penicillin(34) and in 18 African countries within the meningitis belt (2%) reduced susceptibility to penicillin (12) were less than reduced susceptibility isolates of *Neisseria meningitidis* exhibited in this study. However, study reported in Addis Ababa Ethiopia only 4.5% sensitive to Penicillin was higher than reduced susceptibility exhibited **in** isolates of *Neisseria meningitidis* in this study (19)**.** In contrast, study reported in Jimma, Ethiopia isolates were susceptible to penicillin (40).

Reduced susceptible of *Neisseria meningitides* isolates to ciprofloxacin was comparable with study in Addis Ababa Ethiopia of sensitive to ciprofloxacin (83.7%)(19). However, resistance of *Neisseria meningitides* isolates (60%) to Ciprofloxacin in Gurage Zone, Ethiopia was higher than resistant to Ciprofloxacin exhibited in this study(27).

Isolates of *Neisseria meningitides* 84.2% sensitivity to ceftriaxone in this study was comparable with study reported (87%) sensitive to Ceftriaxone in Gurage Zone, Ethiopia (27). The resistance documented in Addis Ababa Ethiopia (69.4%) to ceftriaxone was higher than to ceftriaxone resistance in this study (19).

High susceptibility of Rifampin (94.7%) and Chloramphenicol (97.4) in isolates of *Neisseria meningitides* in this study were comparable with studies reported (7%) resistant to Rifampin and (4%) resistant to Chloramphenicol in Gurage Zone, Ethiopia respectively (27). Resistant to chloramphenicol 3(30%) in Gondar, Ethiopia was higher than higher than the resistant to Chloramphenicol in this study (39).

7 Limitation

In this study serogroupX was not detected because it was impossible to avail serogroupX antiserum during the study period. However, serogroup X was emerging serogroup that was responsible to caused epidemic in the African meningitis belt in recent decade.

8 Conclusions and Recommendation

8.1 Conclusions

Generally, in this study 13.8% participants were carrier of N.meningitidis. The participants harbour most of serogroups that are responsible for invasive cases of meningococcal disease and predominant capsulated isolates were serogroup Y/W135. Low carriage rate of serogroupA was isolated which was previously responsible for the occurrence of epidemic in the meningitis belt. The isolates exhibited resistance to ceftriaxone, penicillin and ciprofloxacin. Respiratory symptom, active cigarette smoking and age group of 16-20 years increase risk of N. meningitidis pharyngeal carriage rate.

8.2 Recommendation

In this study 13.8% participants were carrier of N.meningitidis. The participants harbour most of serogroups that are responsible for invasive cases of meningococcal disease. This study suggests the government to provide for all prisoners drug prophylaxis preventive measure to prevent circulation of Neisseria meningitidis serogroup in the setting of heavily overcrowded living condition. In further, this study suggests the Jimma zonal administration commission to reduce overcrowding commission that fit minimum standard.

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Annex :

Annex: 1 Standard Operation Procedures for Microbiological Technique

A. Media preparation

I. Modified Thayer-Martin Medium preparation (Difco Beton)

1. Suspend 7.2 gm. of GC base medium in 100 mL of distil water and mix thoroughly.
2. Suspend 2 gm. hemoglobin powder in 100 mL purified water to make a 2% solution.
3. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
4. Autoclave at 121°C for 15 minutes then cools to 45-50°C.
5. Aseptically add 100 mL hemoglobin solution 2% to the medium.
6. Aseptically add 2 mL IsoVitaleX Enrichment.
7. Aseptically add 2 mL rehydrated VCNT Inhibitor to the medium.
8. The agar base solution contain the following ingredients:
    1. 3.0 µg/ml vancomycin
    2. 7.5 µg/ml colistin
    3. 12.5 units/ml nystatin
    4. 5.0 µg/ml trimethoprim lactate
9. Dispense 20 ml into 15×100 mm Petri dishes and allow the media to solidify
10. Test samples of the finished product for sterility; incubate not inoculated plate for 48 hours at 35-37°C with 5-10% CO2 (or in a candle-jar).
11. Test samples of the finished product for media a performance using stable, typical control cultures of *N. meningitidis* QC strain for 18-24 hours on a MTM at 35-37°C with 5-10% CO2 (or in a candle-jar).
12. Place the plates in sterile plastic bags and store at 2-8°C until use.

II. Chocolate Agar (HIMEDIA)

1. Suspend 40 gm. in 1 litre of distilled water and bring to the boil to dissolve completely.
2. Sterilise by autoclaving at 121°C for 15 minutes.
3. Cool to 80°C and aseptically add 5-10% sterile defibrinated sheep blood and maintain at this temperature for 5 to 10 minutes, agitating frequently.

4. Test sample of prepared plate for sterility and performance of the medium.
5. Store the prepared medium at 2-8°C.

III. Muller Hinton Chocolate Agar(Accumix)

1. Suspend 38mg in a litter of distilled water and bring to the boil to dissolve completely.
2. Autoclave for 15 minutes at temperature (121 degree Celsius).
3. Let it cool to 80°C and aseptically add 5-10% sterile sheep blood.
4. Dispense 20 ml into 15×100 mm Petri dishes and allow the media to solidify
5. Test samples of the finished product for sterility; incubate an uninoculated plate for 48 hours at 35-37°C with 5-10% CO2 (or in a candle-jar).
6. Test samples of the finished product for media a performance using stable, typical control cultures of *N. meningitidis* QC strain for 18-24 hours at 35-37°C with 5-10% CO2.
7. Place the plates in sterile plastic bags and store at 2-8°C until use.

IV. Muller Hinton Chocolate dilution Agar for penicillin (Accumix)

1. A 38mg of Muller Hinton medium should be suspended in 1 liter distilled water.
2. Bring to heat and let it boil for a minute; just enough for the medium to be dissolved completely.
3. Autoclave for 15 minutes at (121 degree Celsius).
4. Cool to 80°C and aseptically add 5-10% sterile sheep blood.
5. Measure 100mg penicillin and serial twofold dilutions in sterile distil water to make final concentration of 10ug/ml; 5ug/ml, 2.4ug/ml and 1.25ug/ml in 10ml distil water.
6. 1ml of 10ug/ml, 5ug/ml, 2.4ug/ml and 1.25ug/ml antimicrobial agents is incorporated into 19ml base medium of Mueller Hinton chocolate agar for each separate plate.
7. Pour into the sterile petri dish and label the concentration of antimicrobial agents.
8. Test samples of the finished product for media a performance using stable, typical control cultures of *N. meningitidis* QC strain for 18-24 hours at 35-37°C with 5-10% CO2.
9. Place the plates in sterile plastic bags and store at 2-8°C until use.

V. Cystine Tryptic Agar with Glucose, Maltose, Lactose and Sucrose (Difco™)

1. Suspend 28.5 g of the powder in 1 L of purified water.
2. To prepare fermentation medium, add 5-10g of carbohydrate before autoclaving or dissolve medium in 900 mL water, autoclave, and aseptically add 100 mL sterile 5-10% carbohydrate solution.
3. Mix thoroughly and heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
4. Dispense in screw cap bottles or tubes in 6 ml or desired quantity.
5. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes.
6. Test samples of the finished product for performance using stable, typical control cultures.
7. Store the prepared medium at 2-8°C.

VI. Amies Transport Medium with Charcoal (HIMEDIA)

1. Suspend 19.75 grams in 1000 ml purified / distilled water.
2. Heat to boiling to dissolve the medium completely.
3. Dispense in screw cap bottles or tubes in 6 ml or desired quantity.
4. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes.
5. Cool in an upright position. Turn the tubes several times while agar is solidifying, to maintain uniform suspension of charcoal particles.
6. The medium should be kept cool 2-8°C but do not freeze.
7. Prepared bottles of transport medium are not stored longer than 9 months from the date of preparation.

B. Sample Collection and transportation

1. For 8 hours before swabbing, the patient must not be treated with antibiotics or antiseptic mouth-washes (gargles).
2. In a good light and using the handle of a spoon/spatula to depress the tongue.
3. Swab the area using moisten sterile cotton wool swab.
4. Touching the tip of a swab against pharyngeal tonsillar fossa and then passing it in an upward semi-circular motion over the soft palate to the opposite side.
5. Put the swab in to Amie's charcoal transport medium tube.

6. Push the swab down one third of the medium depth and fasten the cap, label the tube and put in cold chain 2-8 oC.
7. Within a hours of collection, deliver the swab with a completed code to the laboratory(50)(6).

C. **Specimen culturing**

1. Work under BSL-2.
2. Inoculate by rolling swab directly on the medium in a large "Z" to provide adequate exposure of swab to the medium for transfer of organisms on a plate of Thayer Martin, Modified agar culture medium.
3. Cross-streak the "Z" pattern with a sterile wire loop.
4. Incubate the plate in a carbon dioxide enriched atmosphere overnight at 35–37oC in candle jar for up to72 hrs.
5. Examining for growth after overnight incubation and again after 48 and 72 hrs.
6. Always check for purity of the growth by performing a Gram stain.
7. *N. meningitidis* is a medium to large, blue-gray, mucoid colony, gram-negative, kidney-bean- or coffee-bean-shaped diplodocus
8. When necessary, make subcultures to ensure purity (6)(50).

D. Oxidase test for the identification of *N. meningitidis*(*Becton, Dickinson and Company)*

1. Use (1% tetra methyl-p-phenylenediamine hydrochloride) oxidase reagent
2. Add a few drops of oxidase test reagent to a strip of filter paper (Whatman No. 1 or equivalent)
3. Pick a portion of colony of fresh isolates (18–24 h cultures) to be tested and rub it onto a treated filter paper with a platinum loop or wooden applicator stick.
4. Use of steel or nichrome loops may cause false-positive reactions.
5. Positive with *N. meningitidis* reactions turn the bacteria violet to purple immediately, or up to 30 s.
6. Negative reactions remain colourless or turn light pink / light purple after 30 second. Delayed reactions should be ignored.
7. The oxidase test aids in the recognition of *N. meningitidis* and other members of the genus *Neisseria*; other, unrelated, bacterial species with cytochrome c in the

respiratory chain (*e.g., Pseudomonas aeruginosa* and *H. influenzae*) are also oxidase positive.

8. Quality Control use positive (Pseudomonas aeruginosa ATCC) and negative (Escherichia coli ATCC) controls run simultaneously*(Becton, Dickinson and Company)*(6)

E. **Carbohydrate utilization by *N. meningitidis***

1. Use cystine trypticase agar (CTA) method.
2. Carbohydrate utilization tests are used to further validate the identification of a strain as *N. meningitidis.*
3. To confirm a culture as *N. meningitidis,* a use four tubes, each containing a sugar (glucose/dextrose, maltose, lactose, and sucrose)
4. With an inoculating needle, collect 2-3 colonies of growth from an overnight culture of *N. meningitidis* on blood agar or chocolate agar.
5. Stab the inoculum several times into the upper 10 mm of medium.
6. Use another sterile needle, or flame the same needle, before inoculating each of the four carbohydrates to be tested.
7. Fasten caps of tubes tightly and place in a 35-37°C incubator (without CO2).
8. Incubate for at least 72 hours (and up to 5 days) before discarding as negative.
9. Development of visible turbidity and a yellow colour in the upper portion of the medium indicates growth and the production of acid and is interpreted as a positive test.
10. Although reactions may occur as early as 24 hours after inoculation, some reactions are delayed.
11. If only glucose or maltose or none of the sugars react, continue incubation for up to 5 days before discarding.
12. Occasionally, strains of *N. meningitidis* are encountered that utilize only dextrose or maltose but not both
13. Result interpretation(6)**.**

Species	**Glucose**	**Maltose**	**Lactose**	**Sucrose**
N .meningitidis	+	+	-	-
N.gonorrhoeae	+	-	-	-
N.sicca	+	+	-	+
N.lactamica	+	+	+	-
M .catarrhalis	-	-	-	-

F. Identification of the *N. meningitidis* serogroup

Serogroup are A, B, C, H, I, K, L, W135, X, Y, Z, and Z' (29E). (Note: serogroup D is no longer recognized) .Groups A and C are the common causes of meningitis outbreaks in Africa, recently W135 and X outbreaks causes while, group B and Y causes endemic meningitis.

1. Dispense 30 µl of sterile physiological solution in the circle on the disposable card.
2. Take 2 to 3 colonies of *N. meningitidis* and carefully emulsify with the loop
3. Gently shake the reagent; holding the dropper bottle(s) upright, place one drop of the appropriate reagent(s) at the periphery of the bacterial suspension on the disposable card.
4. Mix the drop of latex and the sample suspension using a stick.
5. Rotate the card gently
6. Observe for any agglutination visible to the naked eye within a maximum of 2 minutes.
7. Wait for the end of those 2 minutes to conclude a negative result.
8. Quality control: - standard antiserum test for positive polyvalent control latex and latex negative controls and also test for ATCC strain.
9. Laboratorians can work under a safety hood/BSL-2 or above (Pastorex™ Meningitis) (6).

G. Antimicrobial susceptibility testing of N. meningitis

1. Use Penicillin, ceftriaxone, chloramphenicol, ciprofloxacin, and rifampicin.
2. Using a sterile loop, touch the surface of 1 to 4 morphologically similar, isolated colonies grown on a chocolate agar plate incubated in 5-10 CO2-enhanced atmospheres at 35˚C for 18–22 hours.
3. Immerse the applicator into a tube containing sterile saline.
4. Adjust the turbidity of the inoculum to that of a 0.5 McFarland turbidity standard equivalent to 108 CFU
5. Immerse a sterile cotton-tipped swab into the adjusted inoculum.
6. Remove excess liquid by pressing the swab tip against the inside of the tube.
7. Inoculate the entire surface of a 15x150-mm Mueller Hinton + 5% sheep blood agar plate three times with the same swab of inoculum, after each inoculation to ensure even distribution of the inoculum and confluent growth of the bacteria.
8. Use a single swab of inoculum, and do not return the swab to the broth after each rotation.

9. Allow the inoculum to dry on the surface of the plate (which should take approximately 10 minutes).
10. Place the antimicrobial disc onto the agar with sterile forceps and deposit discs so that the centres are at least 24 mm apart , no less than 10 mm from the edge of the Petri dish and nine discs per 150 mm plate or four discs per 100 mm plate
11. If discs have been placed on the agar with other than the self tamping dispensers, press them down with a sterile needle or forceps to make contact with the surface.
12. Dilute 108 CFU in to 1:10 to form 107 CFU for antibiotic agar dilution method.
13. Transfer lul of 107 CFU on predetermined concentration agar plate on spot
14. Incubate the plates in an inverted position in a 5% CO2 atmosphere for 18–22 hours at 35-37˚C. A candle-extinction jar may be used if a CO2-incubator is not available.
15. Because *N. meningitidis* grows well in a humid atmosphere laboratorian may choose to add a shallow pan of water to the bottom of the incubator or add a dampened paper towel to the candle-extinction jar.
16. Quality control(QC) results must be reviewed before reading and interpreting MIZ and MIC USING ATCC strain (6) (49)

Annex: 3 Data Collection Format Instrument (Questionnaire) English Version

Jimma University department of Medical Microbiology data collection format for meningococcal carriage among Jimma zonal prisoners, Jimma Ethiopia

Questionnaire ID No_____Name of prison _____

Room No _____ area _____m2 ,Date _____

Q. No	**Variables**	**Descriptions**	**Remark**
	Socio-demographic factor		
M01	How old are you?	Years_____	
M02	Your sex?	A. Male B. Female	
M03	Geographic area background	A. Woreda_____ B. Kebele_____	
M04	Residential background	A. Rural B. Urban	
M05	What is your educational status?	A. No education B. Primary C. Secondery D. Diploma and above	
M06	How many prisoners live in single room / Shared bedroom with you in this prison?	A. 1-2 B. Above 2 C. Actual count _____	
	Behavioral factor		
M07	How often do you wash your had?	A. soap and water B. water	
M08	Drug abuse/ use in last three months?	A. Passive smoking B. Active smoking C. Khat D. Never	
M09	Did you have incarcerated history before ?	A. Yes B. No	
M10	If Q M09 yes, how many times?	A. One B. two C. three and above	
M11	How long you spent in the prison	Month -----day----------	
	Health related variables		
M12	Did you have previous respiratory symptom in last three month ?.	A. yes B. No C. ----------	
M13	Did you use antibiotic before 14 days within three month?.	A. Yes B. No	

Annex: 3 Guucam ragaa sassabduu (waraqaa Gaaffii) (*Afaan Oromo version)*

Yuunversitii Jimmaa kutaa meedikaala maakiroobaayoolojiittii, guuca ragaa sassabduu sirreefamtoota mana sirreessaa zoonii jiimmaairraa dhukkuba meeninjookokkaal irratti ragaa qorannoof oolu kan sassaabamun,Jimmaa Itiiyoophiiyaa

Lakk. Eenyumma Waraqaa gaaffii_____Maqaa mana sirressaa _____

Kuta_____Bal'ina kutaa_____m2, Guyyaa_____

M01	Umurii	_____	
M02	Koorniiyaa /saala?	A. Diira B. Dhalaa	
M03	Naannawa jiraatan?	A. Aanaa_____ B. Ganda _____	
M04	Bakka jireenyaa?	A. Baadiyyaa B. Magaala	
M05	Haalli baruumsa keetii maal fakkata?	A. Baruumsa hin qabu B. Sadarkaa jalqabaa C. Sadarkkaa lammaffaa D. Dipiloomaafii isa oli	
M06	Mana sirreessa kan kessatti namoota meeqatuu kutaa/ mana cisiichaatokko keessa siwaliin jirata ?	A. 1-2 B. Above 2 C. Lakkoofsan ----------	
M07	Harka kee akkamitti dhiqatta?	A. Samunaa fi bishaan B. Bishaanin	
M08	Dawwa dharra nama qabsiisan Kan tarreefaman kessaa ji'a sadan darban kessatti kam fayyadamte? fayyadamtan?	A. Nama sigaraa xuuxan walin jiradhe B. Sigaraa xuuxe C. Chaatii/jimaa qama'e D. Homaa hin fayyadamne	
M09	Kanan duru mana sirressa galteejirattee beekta?	A. Eyye B. Miti	
M10	Yoo deebiin lakk M09 eyyeen ta'e yeroo meeqaaf?	A. Tokko B. Lama C. Sadiifii isaa oli	
M11	Mana sirressaa kana kessaa hagam turte?	Ji.a_____guyyaa_____	
M12	Ji'a 3 darban kessatti dhukuba qaam harganssuun qabamtee beekta?	A. Eyyee B. Miti C. ----------	
M13	Ji'a 3 darban keessa guyyaa 14 dura dawwaa fayyadamtee beekta?	A. Eyyee B. No	

Annex :3 የናሙናማሰባሰብያ መሳሪያ (መጠይቅ, የቃለመጠይቅ መመሪያ)(Amharic version)

በጅማ ዩኒቨርሲቲ የሕክምና ማይክሮባዮሎጂ ክፍል የማጅራት ገትር ኣምጪ ባክተሪያ በኣፋቸዉ ዉስጥ ከሚገኝባቸው ከጅማ ዞን እስረኞች መረጃ የሚሰበስብበት

መጠይቅ ቁጥር ---------- የእስር ቤትስም ----------

ክፍል_____ስፋት_____ቀን_____

M01	እድሜ?	_____	
M02	ፆታ?	A. ወንድ B. ሴት	
M03	የከዝህ በፊት አድራሻ?	A. ወረዳ _____ B. ቀበሌ_____	
M04	የመኖሪያ ስፍሪያ?	A. ገጠር _____ B. ከተማ _____	
M05	የትምህርት ደረጃ?	A. ያልተማረ/ች B. የመጀመሪያ ደረጃ C. ሁለተኛ ደረጃ D. ዲፕሎማና ከዛ በላይ	
M06	በዚህ እስርበት ዉስጥ በጋር 1ቤት ውስጥ/ በጋራ መኝታ ክፍል ስንት ሰው ይኖራል ?	A. 1-2 ሰው B. ከ 2በላይ C. በቁጥር_____	
M07	እጅህን የምትታጠበው እንደት ነዉ ?	A. ሳሙናእናውሃ B. ውኃ	
M08	ባለፉት 3 ወራት ዉስጥ ከነዝህ የቱን ተጠቅመሃል?	A. ማጨስን B. ከሚያጨሱ ጋር መኖር C. ጫት መቃም D. በጭራሽ	
M09	ከዚህበፊትታስረዋል?	A. አዎ B. አይ	
M10	ተ/ቁ M09 መልስዎ ከሆነ ስንትጊዜ ነው?	A. ለመጀመሪያ ጊዜ B. 2 ኛጊዜ C. 3 ጊዜ እና ከዚያበላይ	
M11	በእስርቤት ውስጥ ያለ የቆይታ ጊዜ	ወር_____ቀን_____	
M12	ባለፉት 3 ወራትውስጥ የመተንፈሻ አካል ህመም ነበርቦት?	A. አዎ B. አይ	
M13	ከ 14 ቀንበፊት 3 ወራት ውስጥ አንቲባዮቲክስን/መድሃኒት ተተቅሞል?	A. አዎ B. አይ	

Annex: 5 Participant information sheet (*English version*)

Jimma University

Name of Principal Investigator: -Samuel Assefa

Title of study:-Prevalence of Meningococcal Pharyngeal Carriage rate and Antibiotic Susceptibility Among Prisoner at Jimma zonal prison in Jimma Town

Introduction: - I am doing research on meningococcal carriage which cause outbreak of meningitidis disease, and very common in Ethiopia, special in high risk group such as closed and crowded population like prison.

I am going to give you information and invite you to participate in this research. You have wright not agree to participate in the research if you feel uncomfortable with. There may be some words that you do not understand. Please ask me to stop as we go through the information and I will take time to explain. If you have questions later, you can ask me,

Purpose: -The purpose of this study to determine the rate of meningococcal carriage and antibiotic sensitivity test among prisoners in Jimma zonal prison.

Benefit of this study: -Pharyngeal asymptomatic meningococcal carriage is common in any health population .It is prerequisite to cause epidemic and fatal diseases in the African meningitis belt including Ethiopian region periodical in dry season. Currently there is limited study conducted on meningococcal carriage in Ethiopia and in high risk group. The information gained on the rate of carriage and antibiotic susceptibility pattern add the knowledge on the study group.

Participant selection: -Because of the nature of causative agent of bacteria is highly transmitted in closed and crowded population even if other risk factors are significant. I assume that the meningococcal carriage rate will be high among prisoner.

I inviting you to take part in this research because it is important to know the burden among the group and I am asking if you would agree to participate.

Voluntary Participation: -Your decision to participate in this study is entirely voluntary. It is your choice whether to participate or not. If you choose not to consent, nothing will change to benefit you gain and you have right to withdraw any time.

Confidentiality: -All information gathered in the course of your participation in this study will be kept confidential and will be only used for your treatment and for research purpose.

Harm: -Possibility of minimal discomfort during tongue depressing and swabbing on pharyngeal plate.

Sharing of the results: -The knowledge that I get from this study will be shared with you before it is made widely available to the public. Confidential information will not be shared.

Who to Contact:-If you have any questions you may ask them now or later, even after the study has started. If you wish to ask questions later, you may contact any of the following:

- **Name of Researcher** -Samuel Assefa
    - Address /telephone No -0910143830 / E-mail-tellask999time@gmial.com
- If you wish to find more about, contact the IRB (Jimma Univesity Review Board)-
    - Address :- Jimma University Ethictical Review Board,Jimma Ethiopia
    - Telephone number/fax :- +251471114484
    - Postal address :- P.O, Box 378 Ethical Review Board ,College of Public Health and Medical Science Jimma Ethiopia

Annex: 5 Hirmatootaaf waraqaa Oddeffanno Kennituu (*Afaan Oromo version***)**

Yuunversiitii Jimmaa:

Maqaa Qorata jalqabaa : Saamu'eel Aseffaa

Mata Duree Qorrannoo:-Sirreefamtota mana sirreessa godina Jimmaa ,magaala Jimma jiran irraa namoota baakteeriiyaa dhukkuba meeniinjookookaali fidun qabanii fi dandeetii dandamanna dawwaabaakteeriichaa baruu ta.a,

Seensa Ani qorranoo kanan gaggessu baakteeriiyaa dhukkuba meeniinjookookaali fidu irratti ta'a. Innis dhukkuba weerarsa meeninjaayitii jedhamu kan dhaqqabsiisufi Itiiyoophiyaa kessatti kan beekamudha ,kessattuu garee midhamtoota ta'an kan akka mana sirreessa bakka nammootni walitti siqaniifii hedduminaan jiratan kessaatti nihammata.

Akka qorannoo kana kessatti hirmaattanif anis odeeffanoo siniifin kenna .Yoo qorannoon kun sinitti tolu bate hirmachuu dhiisuuf mirga gutuu qabdu. Jechoota isiif hingalle yoo jiraatan giddutti nadhaabatii yeroo fudhadhen isiniif ibsa.Yoo dhumaratti gaaffii qabattanis gaaffachuu dandeessu.

Kayyoo:-Mana sirreessaa kana kessatti baakteeriiyaa dhukkuba meeniinjookookaali fidun namoota qabaman fi dandeetii dandamanna dawwaa isaa murtessuu ta'a.

Faayidaan qorrannoo kana:-Laagaa namoota fayyaa ta'an keessatti baakteriyaan dukkuba meniinjookokaalii dhibee osoo hin gessissin akka argamu beekammadha. Kunis battuu baakteriiyaa ta'uun naannawa sabbata Afrikaan meninjitii kan Itiioophiyaas dabalatu keessattii yeroo eeggee waqtii bonaa dukkuba weerara fii ajjeechaaf nama saaxilu kaassuuf akka halduree ta'ee tajaajila. Yeroo ammaa kana itiiyoophiiyaa fii gareewwaa midhamoo ta'an keessattii qorannoon godhame muraasa. Kana beekun immoo raga haraa garee qorannoo kan kessetti nidabala

Filannoo hirmaatotaa :-Haluuma uumama baakteriiyaa ta'ee namootaa walitti siqanii fi baay'ataanii jiraatan keessatti garmalee dadarba .Kanuma irraa ka'uun baakteeriiyaan kun namootaa sirreffamtoota mana sirreessaa keessatti garmalee ni dabala jedheen yaada .

Aniis qorannoo kana kessatti akka qooda fudhatan isinin affeera ,sababni isaas garee kana keessatti fedhii keessaniin barbadanii akka hirmattanin isinin gaaffadha.

Hirmaanna Fedhii Irratti Hundaa'e :-Qorranno kana kessattii hirmachuuf murteessuun kessaan guutumaa guututti fedhii kessan irratti hundaa'a. Hirmachuu fi hirmachuu dhiisuun fedha kessan. Hirmaachuu dhiisuun keessan faayidaa argattaan irratti jijjirama hin fidu. Hirmannaa keessaa yeroo barbaaddan addan kutuu ni dandessu**.**

Iccitii Eeguu :-Odeffannoon yeroo isin hirmaattan isin irraa guurame iccitiin isaa kan eegamee fi yaalaa kessaniifii qorannoo kanaaf qofa kan oolu ta'a.

Hubaatii Qorannoon Geessisu : Yeroo arraaba gadi qabanii fi laagaa irraa jirbiidhaan xaragamu xiqqoo sinitti toluu dhiisu danda'a.

Bu'aa Qorannoo Dabarsuu : Beekuumsa qorranoo kana irraa argame gara ummataatti dabarsuun dura bu'aan isaa dursee isin biraa ga'a .Iccitiin keessaan garu nama biraaf darbuu hin dana'u.

Qaama Dubbistan : Gaaffii kamiyyuu yoo qabaattan amma yookiin booda gaaffachuu ni dandessu.Yemmuu booddee gaaffachuu barbaddan namoota armaan gadii dubbissu ni dandeessu.

- **Maqaa Qorataa**-Saamu'eel Aseffaa
    - Teessoo /Lakk .Bilb-0910143830 / E-mail-tellask999time@gmial.com
- IRB (Jimma Univesity ethical Review committee),
    - Teessoo:-Yuniversiitii Jimmaa,Jimmaa Itiiyoophiyaa
    - Lakk . Bilbil fax :- +251471114484
    - Lakk. Poostaa:- P.O,Box 378 ethics review board ,koollejjii pabliik heelzii fii meedikaal saayinsii, Jimmaa Itiiyoophiyaa

Annex: 5 ለ**ጥናቱ ተሳታፊዎች መረጃ መስጫ ቅጽ (***Amharic Version*)

ጂማ ዩኒቨርሲቲ

የዋና ተመራማሪ ስም - ሳሙል አሰፋ

የጥናቱ ርዕስ:-የማጅራት ገትር በሽታ አምጪ ባክተሪያ ስርጭትና የመድሃኒት ፈዋሽነት መጠን በጅማ ከተማ ውስጥ በሚገኝ በጅማ ዞን እስርቤት የሚደረግ።

መግቢያ:-ጥናቱን የማካሂደዉ በማኒንግኮክሲካል ከሪየጅ/ማጅራትገትር አምጪ ባክተሪያ የማጅራትገትር ወረርሽኝን የሚስከትልና በኢትዮጵያ ውስጥ የተለመደ፣ በተለይ በወህኒቤት አይነት በተቀራረበና በመተፋፈገ ሁነታ ዉስጥ ላሉሰ ዎች። አሁን መረጃን ሰጥዎታለሁና በዚህ ምርምር ውስጥ እንዲሳተፉ ጋብዝዎታለዉ ፣ምኞት የማይሰማዎት ከሆነ በምርምር ላይ አለመሳተፍ ይችላሉ። እርስዎ ያልገባዎት አንዳንድ ቃላት ሊኖሩ ይችላሉ። እባክዎን በመሃል ያስቁምኝና ግዜ ወስድና ለማብራራሎት። ምናልባት መጨረሻ ላይ ጥያቄ ካሎትም መጠየቅ ይችላሉ።

ዓላማ:-የዚህ ጥናት ዓላማ በጅማ ዞን ማረሚያ በእስር በሚገኙ እስረኞች ላይ ያለዉን የማጅራት ገትር አምጪ ባክተሪያ መጠንና የመድሃኒት ፈዋሽነት ሁነታን መወሰን ነው።

የዚህ ጥናት ጥቅም:-በማንኛውም ጤናማ ህብረተሰብ ዉስጥ ይህ ባክተሪያ ይገኛል። ዪህም በደረቃማዉ አየር ዎቅት ኣፍሪካ መኒንጃቲስ በልት ኢቲዮጲያን በሚያጥቅልለዉ ዉስጥ ለሚከሰቱ የማጅረት ገትር ወረርሽኝንና ገዳይ ህመም ቅድመ ሁኔታ ሆነው ይገኛል። በአሁኑ ጊዜ በኢትዮጵያ ከፍተኛ ተጋላጭ የሆኑ ቡድኖች ውስጥ የማጅራት ገትር ተሸካሚና ምዲያኒት ፈዋሽነት ምርመራ ላይ የተካሄዱ ጥናቶች ውስንናቸዉ። ይህ መረጃ ተጋላጭ የሆኑ ቡድኖች ውስጥ አዲስ ወቂት ይጨምራል

የተሣታፊዎች ምርጫ :ከባክቴሪያው ባህሪ የተነሳ ሕዝብ በጣም በተጨናነቀበትና በተቀራረቡ ሰዎች መሃል በከፍተኛ ሁነታ ይተላለፋል። የማጅራት ገትር ባክተሪያ ተሸካሚ መጠን በእስረኞች መካከል ከፍተኛ እንደሚሆን አምናለሁ። በዚህ ምርምር ውስጥ እንዲሳተፉ እጋብዝዎታለሁ፣ ምክንያቱም በቡድኑ ውስጥ ያለውን መጠን ማወቅ አስፈላጊ በመሆኑና እርስዎም ለመሳተፍ እንድስማሙ እጠይቅዎታለሁ።

በፈቃደኝነት ላይ የተመሰረተ ተሳትፎ:በዚህ ጥናት ለመሳተፍ ያደረጉት ውሳኔ በፈቃደኝነትነው። መሳተፍም ሆነ አለመሳተፍ ምርጫው ነው። አለመስማማት ከመረጡ ጥቅሞት ላይ ምንም ለውጥ አይፈጠርም እና ከጥናቱ በማንኛውም ጊዜ ማውጣት ይችላሉ።

ሚስጥራዊነት:-በዚህ ጥናት ውስጥ በሚሳተፉበት ወቅት የተሰበሰቡ ሁሉም መረጃዎች በሚስጢር ይጠበቃሉ እናም ለርሶ ህክምና ለምርምር አላማ ብቻ ጥቅም ላይ ይውላሉ።.

ጉዳት: -ናሙና ስንሰበስብ ምላሶን ስንጫንና ጉሮሮትን ስንጠርግ ትንሽ የምኞት የሚቀንስ ነገር ልኖር ይችላል።

ውጤት ማጋራት:-ከዚህ ጥናት ያገኘሁት እውቀት ለህዝብ ከመበተኑ በፊት ለርስዎ የሚነገር ይሆናል፣ የርሶ ምስጢራዊ መረጃ ለማንም አይጋራም።

ማነጋገር የምትችሉት አካላት:-የምርመራው ጥናት ከተጀመረ በኋላም እንኳ ጥያቄዎችን ወይም ከዚያ ቆይቶ መጠየቅ ይችላሉ። ጥያቄዎችን ኋላመጠየቅ ከፈለጉ ከሚከተሉት ውስጥ አንዱን ማነጋገር ይችላሉ:

- ስም -ሳሙልአሰፋ-
    - አድራሻ / ስልክቁጥር -0910143830/ e-mail-tellask999time@gmial.com
- ስም IRB (ጅማዩንቨርሲትይ RB)ተጨማሪመረጃማግኘትከፈለጉ, ይገናኙ
    - አድራሻ,Jimma University Ethictical Review Board,Jimma Ethiopia
    - የቴሌፎን:- +251471114484

Annex: 6 Consent Form

In one day, within 15 min structured question and sample collection form

1. Name has explained to me what this research is about and why I was asked to be interviewed.
2. I know what this research is about.
3. I have had the chance to ask questions about the research.
4. I know I don't have to be interviewed if I don't want to.
5. I know that if I sign this form I agree to be asked questions and have my answers recorded/written down.
6. If I change my mind during the interview and don't want to be involved.
7. I know I can stop and anything that was recorded or written down will be destroyed.
8. I know my real name will not be used at all during this research.
9. I know that my answers are confidential unless there is reason to believe that I in danger.
10. I know I can contact (Name) at a later date if I have any queries/concerns about the research or what I said, or if I decide I don't want my answers to be used.

Declaration by participant:

I have read, or have had read to me in my first language, and I understand the participant information sheet**. I**_____ consent voluntarily to participate as a participant in this study**.**

Participant's signature_____ Date: _____

Annex: 6 Guca Waliigaltee (Afaan Oromo Version **)**

1. Guyyaa tokkoof,daqiiqa 15 keessatti gaaffiilee bocamanifi kan sampliin ittiin sassabamu.
2. Qorrannoon kun maqaan isaa maal akk ta'efii maaliif akkan gaaffaatamu naaf ibsamee jira.
3. Qorannoon kun maal akka ta'e beeka.
4. Waa'ee qorrannoo kana gaaffachuuf carraa argadhe jira.
5. Yoon hin barbaanne akkaan hin gaaffatamne beeka .
6. Yoon guca kana mallattesse akkan gaaffatamu waligaluufii deebiin koo akka barreefamu beeka.
7. Yeroo gaaffiifi deebee yaada koo yoon jijjire wanti galmaa'ee yookiin warabame hund akka barbada'u beeka.
8. Maqaan dhugaa koo qorannoo kan keessatt akka itti hin fayyadamne beeka.
9. Rakkoo irraa akkan jiradhuu yoo itti amaname malee ,deebiin koo hundi iccitiin akka qabamu beeka.
10. Guyyaa biraa yoon gaaffii qabaadhe ,wa'ee qorannoo yookkin wantaan dubbadhe yookiin deebiin koo akka itti hin fayyadamne yoon barbaade qaaman dubbisu beeka.

Ibsa /Jecha Hirmaataa

Ani afaan koo jalqabaa /oroomootin duubbise yookin naaf dubbifamee fi waa'een odeffannoo kana naaf galee jira .Ani_____ akka hirmaatatti qorannoo kana kessaatti fedhii kootiin walii galee jira.

Mallattoo _____Guyyaa _____

Annex: 6 ለጥናቱ ተሳታፊዎች ዉል ስምምነት (Amharic version)

1. ይህ ስም ይህ ጥናት ምን እንደ ሆነና ለምን ቃለመጠይቅ እንዳደረግሁ ተብራርቶልኛል።.

2. ይህ ምርምር ስለ ምን እንደ ሆነ አውቃለሁኝ።

3. ስለምርምሩ ጥያቄዎችን የመጠየቅ እድል አግኝቻለሁ።

4. ካልፈለግሁ ቃለመጠይቅ እንደማላደርግ አውቃለሁ።

5. ይህን ቅጽ በምፈርምበት ጊዜ ጥያቄዎችን ለመጠየቅ ተስማምቻለሁ እና የእኔ መልሶች የተፃፉ መሆኑን አውቃለሁ።

6. በቃለ-መጠይቁ ጊዜ ሀሳቤን ከቀየርኩ አለመሳተፍ ችላለሁ።

7.በማንኛዉም ግዘ ማቆም እንደምችል አውቃለሁ እናም የተቀረጸ ወይም የተፃፈዉ ማንኛውም ነገር ይደመሰሳል።

8. በዚህ የምርምር ወቅት ትክክለኛ ስሜን ፈጽሞ እንደማይታወቅ አውቃለሁ።

9. እኔን ለአደጋ የሚያበቃ ምክንያት ከሌለ የነመልሶች በምስጢር እንደሚጠብቁ አውቃለሁ።

10.ለላ ቀን በጥናቱ ላይ ጥያቄዎች ወይም የሚያሳስቡ ጉዳዮች ካሉኝ ወይም በኋላ ላይ ጥያቄ ካለኝ ወይም ጥያቄዎቼ እንዳይጠቀሙበት ከወሰንኩ፣ የማናግረዉ ሰዉ (ስም) እና ማግኘት እንደሚችል አውቃለሁ።

ተሳታፊዎች መግለጫ: የተጠየቁትን በመጀመሪያ ቋንቋዬ አንብቢያለሁ ወይም ተነቦልኛል፣ እና _____ የተሳታፊነት የመረጃ ዝርዝር ተረድቻለሁ። በዚህ ጥናት ተሳታፊ ለመሆን በፈቃደኝነት ተስማምቻለሁ።

ፊርማ_____ቀን: _____