ADDIS ABABA UNIVERSITY

COLLEGE OF HEALTH SCIENCES

SCHOOL OF ALLIED HEALTH SCIENCE

DEPARTMENT OF MEDICAL LABORATORY SCIENCES

Prevalence of *Burkholderia Pseudomallei* and other bacterial pathogens in community acquired infections in Tikur Anbessa specialized Hospital and Felege Hiwot Hospital

By

Emawayish Andargie

A research thesis submitted to the Department of Medical Laboratory Sciences, School of Allied Health Science, College of Health Science, Addis Ababa University, in partial fulfillment of Master of Science Degree in Clinical Laboratory Sciences (diagnostic and public health microbiology specialty).

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School of Graduate Studies

This is to certify that the thesis prepared by Emawayish Andargie, entitled:

Prevalence of Burkholderia Pseudomallei and other bacterial pathogens in community acquired infections in Tikur Anbessa specialized Hospital and Felege Hiwot Hospital submitted in partial fulfillment of the requirements for Master of Science degree in Clinical Laboratory Sciences (diagnostic and public health microbiology specialty) complies with the regulations of the University and meets the accepted standards with respect to originality and quality.

Signed by the Examining Committee:

Examiner_____ Signature _____Date _____

Examiner_____ Signature _____Date _____

Advisor_____ Signature _____Date _____

_____

Chairman of the Department or Graduate Program Coordinator

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List of abbreviation

AAU……...Addis Ababa University

AHRI…… Armauer Hansen Research institute

AIDS….….Acquired immunodeficiency syndrome

API……… Analytical profile index

CAP..... .Community-acquired pneumonia

DST……...Drug susceptibility test

EMLA….. Ethiopian Medical Laboratory Association

HIV……Human immunodeficiency virus

PABA……Para-aminobenzoic acid

SOP……Standard Operating Procedures

UTI..... Urinary tract infection

ABSTRACT

Backgroun- *Burkholderia pseudomallei* (*B. pseudomallei*) is a causative agent of a severe and fatal infectious disease which is called melioidosis. *B. pseudomallei*, a Gram-negative bacterium, is an environmental saprophyte found in wet soils. Melioidosis predominantly affects people in regular contact with soil and pooled surface water. The commonest routes of *B. pseudomallei* infection are thought to be inoculation, inhalation and ingestion**.**The disease mostly infects adults with an underlying predisposing condition, mainly diabetes mellitus.

Objective;-the objective of the current study was to assess the profile and susceptibility pattern of *B. pseudomallei* and other pathogenic bacteria from collected clinical specimens of community-acquired infections

Method:-A Hospital based cross sectional prospective study was conducted to determine bacterial profile and antimicrobial susceptibility pattern of pathogenic bacteria from selected clinical specimen from March 2016-June2018in Tikur Anbessa specialized Hospital and Felege Hiwot Hospital in Bahir Dar. During the study period, 315 clinical specimens (either blood, pus, sputum, or urine) from 315 patients were prospectively collected and subjected to microbiological culturing and identification steps.

Result:- Of all the 315 clinical samples collected and subjected to microbiological culturing and identification steps only one isolet (1/315) from pus sample found to be *B. Pseudomallei,* the rest of the clinical speciment were negative for *B. Pseudomallei* characterization. However, around 14 other bacterial strains were isolated from those biological samples. Among the 14 other bacterial isolates 42.6% of them were gram positive and the rest 57.4% isoates were gram positive with predominant isolate 39% of *S. aureus*.

Conclusion:- The current study indicated that the prevalence of bacterial pathogens in the study areas are substancial. Moreover, even if only one isolate of the target organism (*B. Pseudominalei*) is reported from pus sample of a 20 years old farmer, but it is for the first time in Ethiopia.

Keywords: *B. pseudomallei,* melioidosis, Community acquired infection

1. Introduction

1.1 Background

Burkholderia pseudomallei (*B. pseudomallei*) is a causative agent of a severe and fatal infectious disease which is called melioidosis. *B. pseudomallei*, a Gram-negative bacterium , is a water and soil pathogen in Eastern Asia and Northern Australia[1].This disease has emerged over the past 25 years as an important cause of morbidity and mortality in southeast Asia and northern Australia with high incidence, and is also endemic in other tropical regions[2].A number of cases have been reported in Malaysia , Thailand , northern Australia , south China , Taiwan , south India, Africa and America[3]; and it is also increasingly reported from many countries across some countries in Africa including Nigeria, Gambia, Kenya, and Uganda[4].Sporadic cases have been reported in Nigeria, Gambia, Kenya, and Uganda, but the extent of the disease in Africa remains uncertain[5].

*Burkholderia pseudmallei*is an environmental saprophyte found in wet soils[6]. Melioidosis predominantly affects people in regular contact with soil and pooled surface water. The commonest routes of *B. pseudomallei* infection aret hought to be inoculation, inhalation and ingestion[2]. Person-to-person transmission of *B. pseudomallei* seems to be very rare and has only been described few times,such as,between patients and siblings or one of their playmates[7]. Melioidosis primarily affects persons who are in regular contact with soil and water. Infection results from percutaneous inoculation (e.g., by means of a penetrating injury or open wound), inhalation (e.g., during severe weather), or ingestion (e.g., through contaminated food or water)[8, 9]. Vertical transmission [from mother to child] is possible[7] in which it was reported that Melioidosis has been transmitted to infants through breast milk from mothers with mastitis[8].

Melioidosis mostly infects adults with an underlying predisposing condition, mainly diabetes mellitus[6]. Predisposing conditions of acquiring melioidosis include diabetes mellitus, chronic renal failure, immunosuppressive treatments, including steroids, thalassemia, chronic liver disease, chronic lung disease, where one or more of the mentioned conditionsare found in 60–90% of cases[10].It is probably due to immunosuppression mainly cell mediated immunity.

Melioidosis can manifest with a variety of clinical presentations including sepsis, pneumonia, arthritis, and internal organ abscesses, and has been termed “the great mimicker” because it can be confused with a range of diseases. The most notable example is tuberculosis, with an estimated 10% of melioidosis patients presenting with chronic respiratory symptoms and chest radiography mimicking pulmonary tuberculosis[11, 12]. It is also reported that it has enormous clinical diversity, spanning from asymptomatic infection, localized skin ulcers orabscesses, chronic pneumonia mimicking tuberculosis, to fulminate septic shock with abscesses in multiple internal organs. Most diseases are reported from recent infection, but latency with reactivation is described up to 62 years after exposure.Even though most cases are reported from Southeast Asia and northern Australia, melioidosis is increasingly being recognized from people infected in an endemic region. *Burkholderia pseudmallei,* is also considered a potential biologic warfare agent[13].In reported cases, failure of clinical improvement after the administration of anti-tuberculosis drugs led to bacteriological culture of sputum, broncho-alveolar lavage, or blood and the detection of *B. Pseudomallei.* The clinical presentation of melioidosis may mimic tuberculosis; both cause chronic suppurative lesions unresponsive to conventional antibiotics and affect the lungscommonly[14].

Community acquired infection:In reports it was also defined as an infection that occurs in the community or within less than 48 hours of hospital admission and was not incubating at the timm of hospital admission(an infection contracted outside of a health care setting [15].

Community-acquired pneumonia is one of the most common infectious diseases and is an important cause of mortality and morbidity world wide. Typical bacterial pathogens that cause CAP include *Streptococcus pneumoniae, Haemophilus influenzae,* and *Moraxella catarrhalis.*[16].

Host associated and bacterial virulence factors are necessary in the pathogenesis of UTI.The commonest organisms isolated in most *Community acquired*-UTIs are *Escherichia coli* and *Klebsiella species.* Other bacterias isolated from UTI include the *Enterococcus spps , Proteus spps , P. aeruginosa* and *Staphylococci* among others[15]. *Staphylococcus aureus* is also a predominant cause of community-aacquired skin and soft-tissue infection[17].Severe sepsis remains associated with high mortality, morbidity and costs .Microbiological documentation, particularly of blood stream infections, occurs in only a fraction of patients with community-acquired sepsis. Typical bacterial pathogens that cause community-acquired blood stream infections include *E.coli, S.* aureus, and *S.pneumoniae*[18].

1.2. Statement of the Problem

The Gram-negative bacterium *Burkholderia pseudomallei* is the causative agent of melioidosis, a serious infectious disease of humans and animals. Once considered an understood tropical disease confined to Southeast Asia and northern Australia, research on *B. pseudomallei* has recently gained global prominence due to its classification as a potential bioterrorism agent by countries such as the United States and also by increasing numbers of case reports from regions where it is not endemic. Assessing the true global prevalence of melioidosis is challenged by the fact that clinical symptoms associated with *B. pseudomallei* infection are extremely varied and may be confused with diverse conditions such as lung cancer, tuberculosis, or *Staphyloccocus aureus* infection. These diagnostic challenges, coupled with lack of awareness among clinicians, have likely contributed to underdiagnosis and the high mortality rate of melioidosis, as initial treatment is often either inappropriate or delayed. Even after antibiotic treatment, relapses are frequent, and after resolution of acute symptoms, chronic melioidosis can also occur, and the symptoms can persist for months to years[19].

Half of all cases present with pneumonia, but there is great clinical diversity, from localised skin ulcers or abscesses without systemic illness to fulminant septic shock with multiple abscesses in the lungs, liver, spleen and kidneys. At least 10% of cases present with a chronic respiratory illness (sick >2 months) mimicking tuberculosis and often with upper lobe infiltrates and/or cavities on chest radiography. As with tuberculosis, latency with reactivation decades after infection can also occur, although this is rare[11].

Recently, the global environmental distribution of *B. pseudomallei* and the world-wide incidence and mortality of meliodosis was estimated using a modelling approach. It was predicted that 165,000 melioidosis cases occur per year worldwide, in which 89,000 people die . The estimates suggest not only a massive underreporting in countries known to be endemic but also identified 34 countries in which melioidosis is probably endemic and has never been reported. Among those countries are 24 African countries and three countries in the Middle East. Modelling predicts that 24,000 (95% credible interval 8000–72,000) cases with 15,000 (credible interval 6000–45,000) deaths occur annually in sub-Saharan Africa while less than 1000 annual cases and deaths were predicted for North Africa and the Middle East[20].

Melioidosis is third most common cause of death among infectious diseases in northeast Thailand after acquired immunodeficiency syndrome (AIDS) and tuberculosis[21].In Thailand 2000 to 3000 new cases are diagnosed every year. In Malaysia, reported seroprevalence in healthy individuals is 17-22% in farmers (mainly rice farmers) and 26% in blood donors. In north Australia 0.6 to 16% of children have evidence of infection by *B. Pseudomallei*[7] where, 4% of patients present with brain stem encephalitis[6].Similar reports are increasingly coming from other tropical regions. However, melioidosis is under diagnosed in most parts of the world, because microbiological investigations are either not available or the organism is not recognized[22].

Burkholderia pseudmallei considered as a potential emerging infectious agent in many tropical developing countries[20]. Ethiopia being in the tropics and with similar agro ecological condition and soil type with endemic areas we strongly suspects the bacteria may present and cause disease in the country.Death from melioidosis reaches 80% in those who are not treated with effective antimicrobial drugs[23]. Moreover, a report from Steinmetz's laboratory (Germany) indicated that, from soil samples collected on November 2014 from around Bahir Dar town and north of Addis Ababa, *B. pseudomallei* was detected in about 50% of the samples using multiple *B. pseudomallei*-specific qPCR. This Targeted culture attempts led to the isolation of different *B. pseudomallei* strains. This pilot study clearly shows that *B. pseudomallei* do exist in the environment in Ethiopia[24].

Despite the introduction of ceftazidime and carbapenem-based intravenous treatments, melioidosis is still associated with a significant mortality attributable to severe sepsis and its complications. A long course of oral eradication therapy is required to prevent relapse. Studies exploring the role of preventative measures, earlier clinical identification, and better management of severe diseases are required to reduce the burden of this disease[8].

1.3. Significance of the study

Melioidosis is a serious health problem-affecting people of the world. Empirical antibiotic regimens for the treatment of presumed community acquired sepsis, such as penicillin/aminoglycoside combinations, have no activity against this organism and no licensed vaccine exists[25, 26]. Early detection and adequate treatment of melioidosis can reduce morbidity and mortality significantly.

However, in Ethiopia, the prevalence of this deadly disease (Melioidosis) is not known and no sporadic cases of human melioidosis have been reported so far, except one environmental pilot study conducted on soil in Bahirdar and Addis Ababa zuria, which highlighted the presence of *B. pseudomalle*[24]. Further more, increasing reports from Africa warrant the need for further investigation. Although, there is no well-established laboratory method as well as the epidemiology and clinical presentations of melioidosis and the corresponding environmental distribution of virulent *B. pseudomallei* were entirely unknown in Ethiopia. Thus, this study may shade light on the existence of the *B. pseudomallei* in clinical specimens and provides baseline information for further studies in the future.We also believe that, information from this study will provide a clear picture that shows the real status of meliodosis in the country, so as, to conduct a clinical and epidemiological study in a larger scale. The finding of this study will also be in a great use for governmental and non-governmental bodies working on disease prevention and control.

Moreover, the report from this study enriches the existing bacterial profile and antimicrobial susceptibility pattern of other common bacterial isolates, which causes community-acquired respiratory infection, skin infection, sepsis and urinary tract infection.

2. Literature review

Burkholderia pseudomallei, a gram-negative environmental bacterium, is the causative agent of a fatal disease melioidosis.Melioidosis is a life-threatening infection that is estimated to account for ~89,000 deaths per year worldwide.As described over 100 years ago by Alfred Whitmore in Rangoon, the disease is so neglected that it is not even on any of the lists of neglected tropical diseases,despite the fact that it probably kills more people each year than diseases that are much better known, such as leptospirosis and dengue [20].

It was the French colonialist in Indochina first proved that *B. Pseudomallei* was a saprophyte rather than a zoonosis as had originally been suspected. It was discovered for the first time in northern Australia in 1949, although it appears that this is really where it actually originated [27.28]. *B. pseudomallei* appears to have spread from there to southeast Asia, and then to Africa and the Americas [29]. The disease gained brief notoriety as a cause of infection amongst French and American troops serving in Southeast Asia. Its unusual ability to remain latent after acquisition and cause a fatal disease many years later has given rise to the nickname 'Vietnam Time Bomb' [28]. More recently, the Centers for Disease Control and Prevention (CDC) has categorised *B. Pseudomallei* as a 'Tier 1 Select Agent'because of its biothreat potential, resulting in increased research and understanding of melioidosis.

Burkholderia pseudomallei, as an important biothreat agent whose differentiation from its near-neighbor species is always a challenge. This is because of its phenotypic similarity with other Burkholderia species which have a wide spread geographical distribution with shared environmental niches. Globally, there are where melioidosis is highly endemic, endemic, or sporadic and possibly endemic are indicated (Fig.1) It is a major public health concern especially, in endemic regions including Southeast Asia and northern Australia. Currently, the known global distribution of melioidosis is expanding, a reflection of improvements in diagnostic microbiology and increasing numbers of cases in travelers and returning military personnel [6]

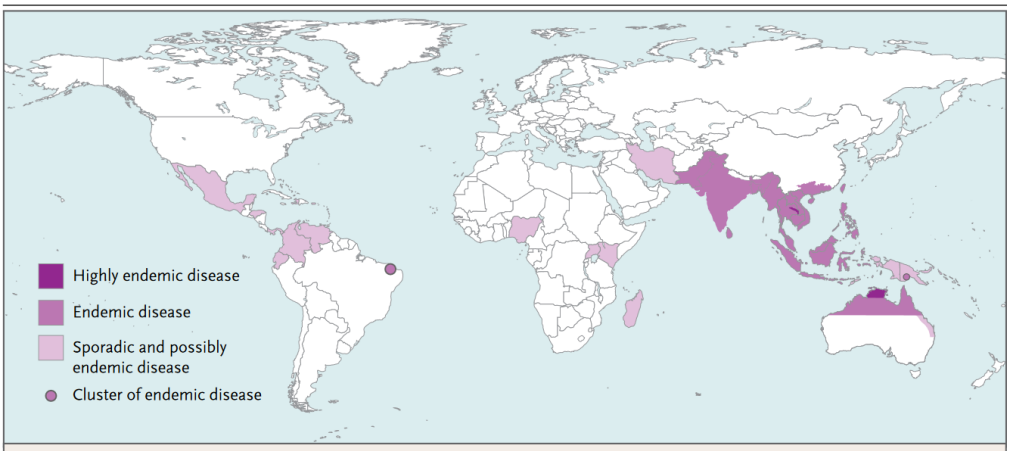


Figure 1. Global Distribution of Melioidosis.
Areas where melioidosis is highly endemic, endemic, or sporadic and possibly endemic are indicated. This reflects current knowledge that is based on limited evidence and is likely to change over time.

Source :Wiersinga WJ, Currie BJ, Peacock SJ.Melioidosis.*N Engl J Med* 2012;367:1035-44.

Although, prevalence surveys of this saprophytic bacterium in environment are under-reported in the some countries, but there are ample of reports from studies conducted at different clinical settings indicating the isolation rate of *B.pseudomallei*. For instance, a study conducted inThailand compared the isolation rates of *B.pseudomallei* from various clinical specimens at different community Hospitals located in the central, north, northeast, and south of Thailand indicated that, the isolation rates of *B. pseudomallei* were 4.2 and 4.1 per 1,000 clinical specimens in northeastern hospitals as compared to 1.1and 1.8 in central, 1.1 and 1.1 in north,and 1.2 and 0.7 in south Thailand[30]. Similarly, a cross-sectional study conducted at Udon Thani Hospital, northeast Thailand, enrolled 118 patients with suspected pulmonary tuberculosis,also indicated that 3 out of 118 TB suspected patients were sputum culture positive for *B. pseudomallei*[31].

A prospective study of melioidosis was carried out in northern Australia, 252 cases were found over 10 years. Of these, 46% were bacteremia and 49 (19%) patients died. Despite administration of ceftazidime or carbapenems, mortality was 86% among those with septicshock. Pneumonia accounted for 127 presentations (50%) and genitourinary infections for 37(15%), with 35 men (18%) having prostatic abscesses. Other presentations included skin abscesses (32 patients; 13%), osteomyelitis and/or septic arthritis (9 cases; 4%), soft tissue abscesses, and encephalomyelitisshowed similar percentages (10 cases; 4%)[32].

There are few data on paediatric melioidosis in endemic areas outside rural north-eastern Thailand and northern Australia. This study reports 16 culture-confirmed cases of melioidosis in children aged 15 years seenbetween 1976 and 2005 at an urban teaching hospital in Kuala

Lumpur, Malaysia. Seven (43.8%) patients had septicaemic melioidosis (with three known deaths) and nine (56.2%) had localised disease (one death). Eleven (68.8%) patients had underlying diseases, including five with haematological malignancies. Skin, soft tissue and lymph nodes were most commonly affected[33].

Retrospective study condacted in Pahang, Malaysia included patients < or =18 years old with positive body fluid cultures for Burkholderia pseudomallei from January 2000 to June 2003. Data on culture results were obtained from 2 referral hospitals. The incidence of pediatric melioidosis was 0.68/100,000 population per year[34].

In 1985 the first documented human infection with *Pseudomonas pseudomallei* in West Africa was documented. A 12-year-old girl from Sierre Leone was admitted to the Medical Research Council Unit in Fajara, after admission she had a osteomyelitis and developeda left palmer abscess, from which *B.pseudomallei*was isolated[35].

In Mauritius a 40-year-old patient was admitted to the hospital on January, 2004, with sepsis and cellulitis. This was the first time *B. pseudomallei*was isolated in Mauritius. The patient was immunecompromised, had never traveled abroad, and had a history of regular exposure to mud. *B. pseudomallei* was identified by API 20NE after it was isolated in pure culture from blood. Antimicrobial susceptibility testing bydisc diffusion showed resistanceto colistin, ampicillin, cephalexin, gentamicin, and ciprofloxacinand susceptiblity to co-amoxiclav, tetracycline, cefotaxime,ceftriaxone, ceftazidime, piperacillin, and meropenem. Alarge zone of inhibition was seen around the co-trimoxazoledisc[36].

A first case of melioidosis imported from Madagascar in 2004 was recognized in La Réunion, a French overseas department, located east of Madagascar. A 58-year-old man with Pneumonia was reported in 2006 which is second case of melioidosis probably imported from Madagascar. The patient, spent most of his life in France. He traveled for short periods in Tunisia, Turkey, and Mauritius (6 years ago) and had been living in Antananarivo, Madagascar, for the past 5 years. The culture of bronchoalveolar lavage came positive for B pseudomallei, by use of the API 20 NE identification system[37].

In October 2009, imported melioidosis case was found in Spain from a 29-year-old Gambian immigrant with diabetics who visited West Africa during the rainy season.The patient had pyomyositis and pneumonia. Culture from the pus aspirated from the cough and several sputums of the patient produced a pseudomonal-like microorganism and confirmed as

Pseudomonas pseudomallei by API 20 NE. The microorganism was sensitive to ceftazidime, cefepime,amoxicillin/clavulanate,piperacillin,piperacillin/tazobactamandchloramphenicol[38]

Another case in 2009 was ,a 60-year-old African man, presenting Melioidosis as mycotic aneurysm(the cause of vascular, lung and liver melioidosis) was admitted to the hospital of Francce ,*B. pseudomallei* was isolated from culture (blood, arterial tissue)but he had been in multiple countries in the continent or the specific country of acquisition was not detailed . The patient was treated with Imipenem and ciprofloxacin for 5 weeks, followed by oral antibiotic therapytrimethoprim–sulfamethoxazolefor 5 months and queried from the disease[39].

A 35-year-old previously healthy woman was admitted to the emergency unit of Ramón y Cajal University Hospital, Madrid, Spain, on March 2011, because of Sepsis. She had just returned from an 11-month leisure travel trip through Africa, during which she visited Madagascar and 14 countries in West Africa. *B. Pseudomallei* was isolated in pure culture from blood. Exposure was in multiple countries on the continent or the specific country of acquisition was not detailed. Before identification of the bacteria the patient was treated with intravenous ceftriaxone; oral doxycycline was added the next day,but acute progressive dyspneawas observed.After identification of the bacteria the treatmentwas changed to intravenous ceftazidime andoral doxycycline, and the patient showe drapid clinical improvement. Later changed to oral cotrimoxazole for 3 months course and completely recovered from the disease[40].

In March 2011 a 16-month-old boy was seen at Queen Elizabeth Central Hospital (QECH), Blantyre, Malawi with Sepsis, Cutaneous abscesses, of human melioidosis .He was given chloramphenicol for empirical treatment of systemic bacterial infection before the isolate was identified but he did not respond to the treatment.*B. pseudomallei* infection was diagnosed, and treatment was changed to IV ceftazidime then he recovered from the disease [41].

Some cases of Autochthonous Melioidosis in humanswere foundin Madagascar, one of the cases werea 52-year-old male rural rice farmer, who had diabetes. He was admitted to Androva University Hospital in Mahajanga in July 2012 with systemic sepsis, from which he died 3 days later despite treatment with ceftriaxone. Abdominal ultrasound showed hepatomegaly and splenomegaly with small hypoechogenic lesions in the spleen consistent with abscesses. A blood culture taken the day after admission was positive for *B. pseudomallei* which was suspected 8 days later on the basis of a biochemical phenotype using API20NE.

The second case was a 45-year-old male rice, sugar cane, and tobacco farmer, who had diabetes. He was admitted to Androva University Hospital in May 2013 with a recurrent fever and a history of furunculosis for several months. A week after admission, his condition deteriorated; he had progressive sepsis and hepatic failure despite therapy with ceftriaxone, ciprofloxacin, metronidazole, and gentamicin. Ultrasound of the abdomen showed hepatomegaly with a multinodular appearance and splenomegaly. Four blood samples were cultured on different days during his illness. The identification of *B. pseudomallei* was done using API20NE. After melioidosis was presumptively diagnosed, the patient was immediately treated with ceftazidime but he died 24 hours later, 2 weeks after admission[42].

Retrospective analysis of 158 confirmed cases of melioidosis collected from medical records from 2001 to 2015 in Hospital Universiti Sains Malaysia, Kubang Kerian, Kelantan, Malaysia.patients were lung infection in 65 (41.1 %), skin infection in 44 (27.8 %), septic arthritis/osteomyelitis in 20 (12.7 %) and liver infection in 19 (12.0 %) . Internal organ abscesses and secondary foci in lungs and/or soft tissue were common. A total of 67 (41 %) cases presented during the monsoonal wet season[43].

Study condacted in south East Asia and Iran on *Isolation of B. pseudomallei* from the soil, 12 strains of *B. pseudomallei* were isolated from the soil and water of a sheep paddock over a two-year period. The organism was recovered from the clay layer of the soil profile as well as from water that seeps into this layer during the "wet" season. Environmental factors appearto play an important role in the survival of *B. pseudomallel* during the"dry" season. Lower Isolation rates were recorded than those indicated by workers in southeast Asia and Iran[44].

Review to summarizes the geographical distribution and clinical impact of melioidosis, especially in the tropics.Several cases have been reported from different regions of India.For example, a noticeable epidemic of plaguelike illness was caused by *B. pseudomallei* ,which was later clinically confirmed as melioidosis . Chronic melioidosis has also been reported in cystic fibrosis patients of Indian origin . In India, melioidosis has acquired the status of a newly upcoming transmittable disease[45].

Review done in Melioidosis in Africa , environmental studies revealed the presence of *B. pseudomallei* in soil samples in various regions of the Ethiopia using molecular as well as cultural methods. The genomes of isolated environmental Ethiopian *B. pseudomallei* strains are currently being analyzed [24].

A cross sectional study condacted in addis ababa ,Ethiopia , Out of the 201 tested blood samples, blood cultures were positive in 56 (27.9%).Gram negative and Gram positive bacteria constituted 29(51.8%) and 26(46.4%), respectively. The most frequent pathogen found was *Staphylococcus aureus* 13 (23.2%), followed by *Serratia marcescens* 12(21.4%), *CoNS* 11(19.6%), *klebsiella spp* 9(16%) and *Salmonella spp* 3(5.4%). Majority of bacterial isolates showed high resistance to Ampicillin, Penicillin, Co-trimoxazole, Gentamicin and Tetracycline which commonly used in the study area[46].

A retrospective cross-sectional study was conducted in the University of Gondar Teaching Hospital from March to May 2013 .From a total of 856 bacteremia-suspected cases, 169 were positive. Mixed bacterial growths were observed in five specimens so that a total of 174 bacteria were isolated . From the isolated bacteria, Gram-positive bacteria were more prevalent due to staphylococci (CoNS) than Gram-negative bacteria. The most commonly isolated bacteria were coagulase-negative staphylococci (CoNS) followed by *S. aureus*, *E. coli*, and *Citrobacter* species[47].

A prospective cross sectional study was conducted on Desse Regional Health and Research Laboratory (DRHRL) that serve outpatients in the north east Ethiopia (south wollo) on Urinary tract infection (UTI), From 156 urine specimens, bacterial isolates were found in 49 (31.4%). The most common pathogens isolated were Escherichia coli (55.1%), Klebsiella spp. (16.3%), Proteus spp. (12. %) P. aeruginosa (4.1%) S. aureus (6.1%), Enterococcus spp. (4.1%) and Citrobacter spp. (2.0%). All isolates in the study showed high rate of resistance to ampicillin, tetracycline, penicillin, vancomycin, cloxacillin and amoxicillin (>72.9%)[48].

3. Objectives

3.1. General objective

- To assess the profile and susceptibility pattern of *B. Pseudomallei* and other pathogenic bacteria from collected clinical specimens of community-acquired infections (an infection contracted outside of a health care setting or an infection present on admission).

3.2. Specific objectives

- To isolate and identify *B.pseudomallei* from different clinical specimens(urine, blood ,pus and sputum) using various culture medium including ashdown agar.
- To identify the spectrumof other bacterial pathogens isolated from different clinical specimens(urine, blood ,pus and sputum)
- To determine the antimicrobial susceptibility pattern of commonly isolated clinical strains.

4. Materials and methods

4.1. Study area

The study was conducted in Tikur Anbessa specialized Hospitaland Felege Hiwot Hospital in Bahir Dar, Ethiopia.

4.2. Study design and study period

A Hospital based cross sectional prospective study was conducted to determine bacterial profile and antimicrobial susceptibility pattern of *B. pseudomallei* and other pathogenic bacteria from selected clinical specimens from March2016-June2018.

4.3. Population

4.3.1 Source Population

All attending the selected hospitals during the study period.

4.3.2. Study population

All patients with respiratory, skin,sepsis, and urinary tract community acquired infection,and visiting Tikur Anbessa specialized and Felege Hiwot Hospitals,who were agreed to enrolled in the current study and have not taken antibiotic before 48 hourswere screened for bacterial profile.

4.4. Variables of the Study

4.4.1. Dependent variables:

- Bacterial profile isolated from sputum, urine,blood and pus.
- Drug susceptibility pattern of all isolates.

4.4.2. Independent variables:

- Socio-demographic characteristics (Age,sex, address, occupation)
- Specimen type

4.5. Inclusion and exclusion criteria

Inclusion criteria

- All Patients who present with clinical symptoms of community acquired respiratory disease, skin infections, sepsis and urinary tract infection on admission or develop respective symptoms with in 48 hours after admission

- Patients not treated with any antibiotic before 48 hours.If treated not by ceftazidime or carbapenems**.**

Exclusion criteria

- Patients unable to give consent to participate in the study.
- Patientswho have been treated with ceftazidime or carbapenems and were not able to suspend the treatment for 48 hours.
- Patients,admitted in hospital formore than 48 hours during the study period.

4.6 .Sample size determination and Sampling technique

4.6.1. Sample size determination

Since there was no related literature on the current topic, we determined our sample size by considering a 50 % prevalence using Dannie's formula

$$\mathbf{n}=\frac{(Z\alpha/_2)^2 * p(1-p)}{d^2}=\frac{(1.96)^2 * 0.5(1-0.5)}{(0.05)^2}=\frac{(3.84*0.25)}{0.0025}=384$$

Where **n** stands for the estimated sample size, **d** forexpected margin of error which is 5%=0.05, Zα/2 for 95%confidence interval (C.I) =1.96, whereas p for proportion of occurrence of the event. Considering a 10% contigency the minimum sample size for this study was 384. But we achived to collect only 315 samples within a given time frame.

4.6.2. Sampling procedures/technique

convenient sampling technique was employed to include study participants,who were attending the bacteriology units of the two Hospitals, as per the inclusion criterion. All samples processed at the study sites, while samples with suspected isolates of *B. Pseudomallei* were further analyzed in the bacteriology laboratory of Armauer Hansen Research Institute (AHRI).

4.7. Data management and Quality control

Data quality was ensured by using standardized data collection materials, pretesting of the data collection sheet, proper training was given to all data collectors before the start of data collection, and intensive supervision during data collection by the principal investigator. During laboratory analysis pre-analytical, analytical and post-analytical stages of quality

assurance which is incorporated in the Standard operating procedures (SOPs) of the microbiology laboratory of AHRI was strictly followed. In addition, well-trained and experienced laboratory professionals were participate in the laboratory analysis procedure.

Pre-analytical phase

At this stage, we mainly considered all steps of sample collection. For instance, for patients with UTI we strongly recommend midstreamurine collected using sterile containerfor microbiological culture, because of the reduced incidence of cellular and microbial contamination.Blood cultures were taken after clinical identification of possible bacteremia or sepsis and before the administration of antibiotics. Pus swabs were aseptically obtained using sterile cotton from wound sites before the wound is cleaned by antiseptic solution.Following collection, all specimens were transported to the microbiology laboratory within 30 minutes.

Analytical phase

At this stage of quality assurance all materials, equipment and procedures were adequately controlled. Culture media for its sterility, growth performance, stability andpH value were tested.To standardize the inoculum density of bacterial suspension for the susceptibility test,a McFarland standard equivalent to 0.5 was used. Standard reference strains were also used as control bacterial strains.During all these steps a Standard operating procedures (SOPs) of the microbiology laboratory of AHRI were strictly followed, and all the results were checked and approved by the supervisors

Post-analytical phase

At this final stage of quality assurance, we strictly followed the recording and documentation steps. For instance, we recorded the laboratory results with the patients' identification number. In addition, in order to avoid the errors in the results of the test, every report checked twice before the results given to the respective hospitals.

4.8. Data Processing and Analysis

Data analysis was done using STATA statistical software. A descriptive statistics was calculated & logistic regression analysis was used to see the relation between dependent variable and independent variables. The association was assessed by using chi-square test. Variables that was show a significant association was selected for further analysis. In all cases

P-value, less than 0.05 was considered as statistically significant. The strength of the association was interpreted using an odds ratio in a 95% confidence interval. Finally, tables and words used to depict the results.

4.9. Ethical consideration

The current research project was ethically cleared by the Ethics and Research committee of Addis Ababa University, College of Health Science, School of Allied Health Science and Department of Laboratory Sciences, and AHRI/ALERT. In addition, all study participants were recruited after they informed about the objectives of the study and gave informed consent. There were minimal risk associated with the process of sampling; it is the same as taking specimen for culture and sensitivity in the routine laboratory diagnosis. For all confirmed clinical samples, the responsible clinicians of the subject's were informed. All the information contained within the study was kept confidential.

4.10. Dissemination of results

The study report will submitted to Addis Ababa University, College of Health Science, School of Allied Health Science and Department of Laboratory Sciences. The reportwill also be submitted to research and publication office of AHRI, Tikur Anbessa specialized Hospital and Felege Hiwot Hospital.The paper will submitted to international or national peer reviewed journal for publication.

4.11. Data collection procedures

I. Demographic characteristics and exposure to risk factors

Information regarding demographic characteristics of the study participants as well as exposure to potential risk factors were collected using a pre-structured questionnaire.

II. Specimen collection and transportation

Informed consent from adults and assent from participants whose age is below18 yearsold were obtained from the guardian before sample collection and enrollement in the study. During the study, different types of clinical samples, such as pus, blood, urine and sputum for routine patient diagnosis were used. (Annex 6)

Pus: A piece of the infected tissue was taken as the best specimen.However, in cases from which this could not be achieved, pus samples from the wound taken as the best specimen.Since many microbial floras live on skin and mucous membrane, maximum care was taken to not touch the surrounding of the wound.Therefore, the assigned physician were first clean the surface of the wound using 70% alcohol before a wound swab was taken.In addition, aspirates of pus samples from the wound were collected using syringe.Immediately after collection, the samples were sent to the microbiology laboratory in a sterile container.

Blood sample: A 3-5ml blood sample was collectedfrom indicated participants for cultureafter proper skin disinfection of the collection site with 70% alcohol to minimize contamination with skin bacteria.

Urine sample: The patient was instructed to collect a clean-catch midstream urine sample for testing. This method helps protect the urine sample from contaminant microbial floras around the genital area.

Sputum: sputum samples that were coughed up into a sterile cup provided by the laboratory. Deep coughing was generally required, and each participant who were intended to provide sputum were clearly informed that it is phlegm/mucus from the lungs that is necessary, not saliva.

Transportation of specimens: following collection from patients, specimens were transported in sterile container or tube to the microbiology laboratory within 30 minutes.

III.Sample Processing

Following collection, clinical specimens came from non-sterile site were inoculated into Ashdown agar and incubated at 37-42°C for up to 96hours. The clinical samples were also inoculated into different culture media depending on sample type at the same time.Positive cultures were identified by their characteristic appearance on their respective media, Gram reaction (Annex.6) and further confirmed by the pattern of biochemical reactions using the standard method[49].

Biochemical tests: A panels of biochemical tests were performed on colonies from primary cultures for final identification of the isolates.A gram negative, oxidase positive, non-lactose

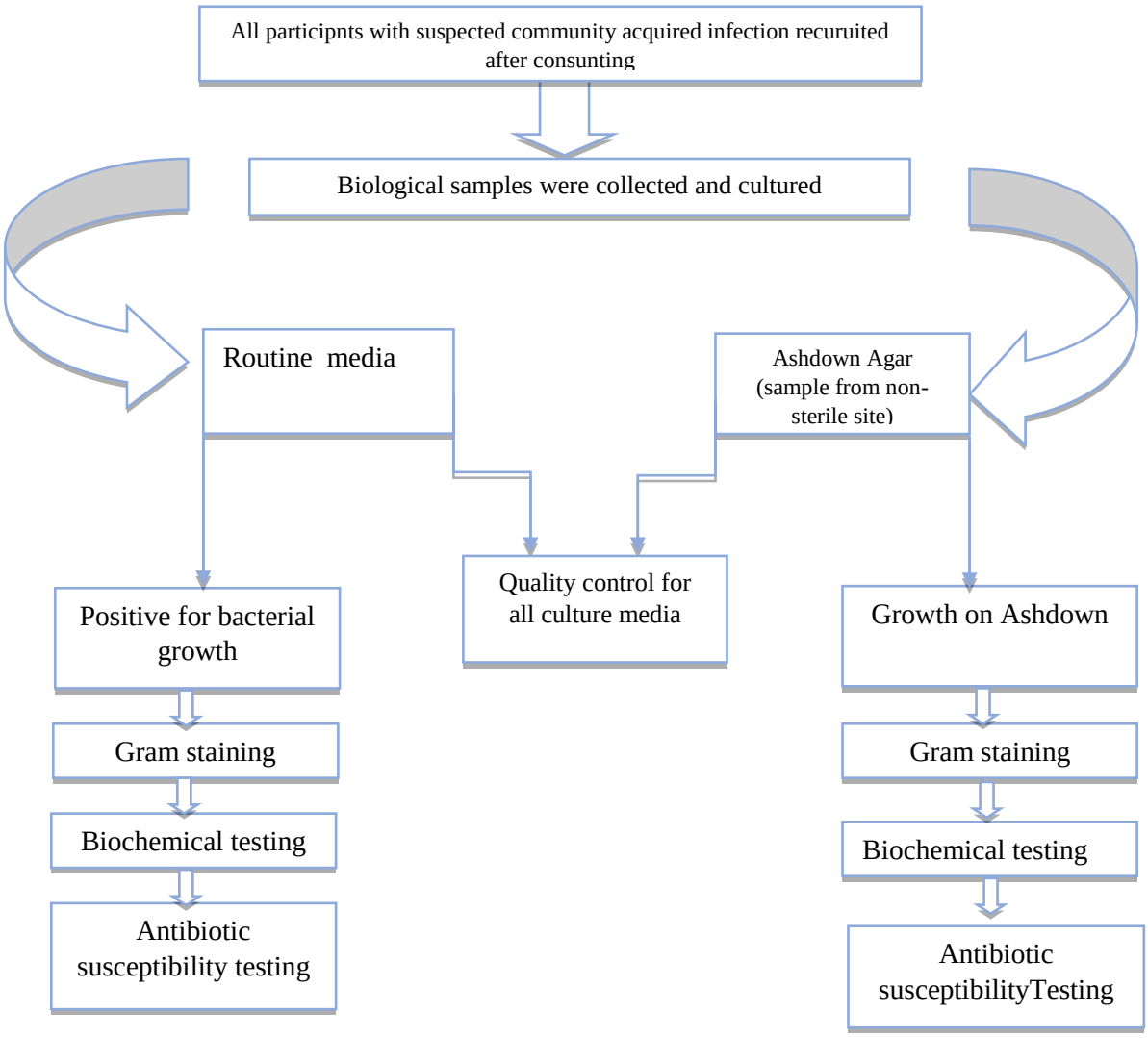
fermenter, Indol and urease negative, citerate and catalase positive were characteristics of *P. aeruginosa*. Any Gram negative, oxidase positive,non-lactose fermenting rod shape bacteria that was not *P. aeruginosa,* grown on Ashdown agar or any other medium (also at 42°C) and was resistant to colistin and susceptible to amoxicillin/clavulanic acid was identified as *B. pseudomallei* (Annex.6).

Antibiotic susceptibility test: Only the conventional antibiotics commonly available for frequent use in the study area were considered for the current study. The drugs, which was tested for both gram negative and Gram positive bacterial isolates were ceftazidime,ciprofloxacin, gentamicin, cotrimoxazole, chloramphenicol, doxycycline, tetracycline,cephalotin, cefotaxime, nitrofurantonin, ampicillin, ceftriaxone, Vancomycin, clindamycine, pencilin and erythromycin.

Antimicrobial susceptibility tests were done as per the standard operation procedures on Mueller-Hinton agar using Kirby Bauer disk diffusion method developed by Bauer et al. The suspension of the test organism was prepared by picking parts of similar test organisms with a sterile wire loop, suspended in sterile broth and was incubated up to two hours to allow organisms reach their log-phase in growth. Turbidity of the broth culture was equilibrated to match 0.5 McFarland standards. The test organism was uniformly seeded over the Mueller-Hinton agar surface and exposed to a concentration gradient of antibiotic diffusing from antibiotic impregnated paper disk into the agar medium. The medium was incubated at 37ºC for 18-24 hours. Then, each bacterial strains were classified into three groups: sensitive (S), intermediate (I) and resistant (R) as indicated in the manufacturers guide[50].(Annex.7).

V. Work flow

1. Work flow for clinical specimen processing



5. Result

Socio-demographic characterstics

A total of 315 patients suspected with community aquired infections by convenient sampling technique were enrolled in the current study. Male accounted 157 (49.84%) and females 158(50.16%), with ranges of age < 12 years were 81(25.71%) patients; 12-18 years were 28(8.89%) patients and >18 years were 206(65.4%) patients. About 64 (20.32%) of the patients were farmers and the rest of the patients, 251(79.68%) have different occupation. More than half of the participants 161(51.1%) were from rural areas (Table-1).

Table 1: Socio Demographic distribution of patients with community acquired infections at Tikur Anbessa specialized Hospital and Felege Hiwot Hospital, from March 2016-June2018.

Demographic characteristics		Number	Percentage
Age	<12	81	25.71
	12-18	28	8.89
	>18	206	65.4
	Total	**315**	**100**
Address	Rural	161	51.1
	Urban	154	48.9
	Total	**315**	**100**
Occupation	Farmer	64	20.32
	Other	251	79.68
	Total	**315**	**100**
Sex	Female	158	50.16
	Male	157	49.84
	Total	**315**	**100**

Prevalence of culture confirmed community acquired infections

Distribution of *B.pseudomallei* isolates

All the clinical specimenwere passed through the routine bacteriological culturing process, gram reaction and biochemical tests, so as, to detect and isolate the target

organism, *B.pseudomallei*. Unfortunatlly, from the four types of clinical speciment only one isolate of *B.pseudomallei* from pus sample which fulfilled the craitera of identification of *B.pseudomallei*. All the other clinical specimens (blood, sputum and urine) were negative for bacteriological characterization of *B.pseudomallei.*(Table-.4)

Distribution of *Burkholderia pseudmallei* and other community acquired infections

During the study period patients were divided into 3 age groups. According to the specimen cultured result, the prevalence of infections were almost equal among the patients with age-group between 12-18 years old (25%) followed distribution among patients with age >18years old (24%), and the least distribution was among younger patients aged<12 years old (23%); P-value=0.986. Exposure to is relatively higher among males (29%) than women (18%), with p-value P= 0.022. The result of this study also showed that participants from rural areas (26%) are more affected by *B. pseudomallei* and other bacterial patogen than those from urban areas (22%), with a P-value of 0.322. The rate of exposure status per participants occupation indicated that the prevalence of *B. pseudomallei* and other bacterial patogen were higher (28%) among farmers than participants with different occupations (23%), with a P-value of 0.364(Table-2).

Table 2: Culture confirmed community acquired infections in relation to the socio-demography characteristics of patients from Tikur Anbessa specialized Hospital and Felege Hiwot Hospital,from March 2016-June2018.

Variables	Culture		P-value
	Positive (%)	Negative (%)	
Age : <12	0.23	0.77	P=0.986
12-18	0.25	0.75	
>18	0.24	0.76	
Sex : F	0.18	0.82	P= 0.022*
M	0.29	0.71	
Address : urban	0.22	0.78	P= 0.322
Rural	0.26	0.74	
Occupation: Farmer	0.28	0.72	P=0.364
Other	0.23	0.77	
Specimen type: Urine	0.20	0.80	P= 0.027*
Blood	0.21	0.79	
Sputum	0.15	0.85	
Pus	0.41	0.49	

Proportion of culture confirmed infections by speciemen type

Of the 315 collected biological samples 175(55.55%),43(13.65%), 7 (2.22%) and 90 (28.57%) were blood, pus, sputum and urine respectively. The positivity rate of those samples for community aquired bacterial pathogens was 38(21.71%),18(41.86%), 1(14.29%) and 18(20%) for blood, pus, sputum and urine respectively. This means from the total 315 biological samples, 75 (23.81%) samples were culture positive(Table-3).

Table 3: Culture results of samples collected from patients with community aquired infections at Tikur Anbessa specialized Hospital and Felege Hiwot Hospital from March 2016-June2018.

Specimen collected	Culture result		Total
	Negative	Positive	
Blood	137 (78.29)	38 (21.71)	175(55.55)
Pus	25 (58.14)	18 (41.86)	43 (13.65)
Sputum	6 (85.71)	1 (14.29)	7 (2.22)
Urine	72 (80)	18 (20)	90 (28.57)
Total	240 (76.19)	75(23.81)	315 (100)

P=P= 0.027*

Distribution ofbacterial isolates by speciemen type

During our analysis from the 4 types of biological samples more than one microorganism were isolated, that is 14 bacterial strains were isolated. Of the isolates, 42.6% bacterial strains were Gram positive and 57.4% strains were Gram negative. The detail isolated strains from each biological specimens indicated that from blood 21(28%) isolates were gram positive and 17(22%) isolates were gram negative; from pus 10(13.30%) isolates were gram positive and 8(10.7%) isolates were gram negative; from sputum 1(1.3%) isolates were Gram negative; and from urine1(1.3%) isolates were Gram positive and 17(22.7%) isolates were Gram negative.Of the total isolates detected in all samples *S. aureus*18(24%) from blood and 10(13.3%) from pus was the most frequent one followed by *K.pneumoniae*,6(8%) from blood and 5(6.7%) from urine, while *S.aureus* (from urine), *K.pneumoniae* (from pus and sputum), *P. Aeruginosa*(from blood), *Citrobacter spp*(from blood, pus and urine), *Acinetobacter spp*(from pus), *Enterobacter Spp.* (from urine),*S.arizona*(from blood), *p.vulgaris*(from blood and urine), *B. pseudomallei*(from pus) *and Klebsiella spp.*(from pus) were being the least frequent, with only 1 (1.3%) isolated strains. From the total samples of all kind only one

straine *1(1%)* of *K.peneumoniae isolate* was isolated from sputum.One of the most important finding of the current study was the detection of one isolate of *B.psuuedomalei* from pus sample(Table-4).

Table 4: Distribution of bacterial isolates by speciemen types collected from patients with community acquired infections at Tikur Anbessa specialized Hospital and Felege Hiwot Hospital, from March 2016-June2018.

Bacterial profile	**Specimen type**				
	Blood	Pus	Sputum	Urine	Total
S.aureus	18(.24)	10(.133)	-	1(.013)	29(.386)
Enterococcus spp	3(.04)	-	-	-	3(.04)
Subtotal Gram positive	**21(28%)**	**10(13.3%)**	-	**1(1.3%)**	**32(42.6%)**
K.pneumoniae	6(.08)	1(.013)	1(.013)	5(.067)	13(.17)
E.coli	-	3(.04)	-	5(.067)	8(.1)
Pseudomonas spp	-	-	-	1(.013)	1(.013)
P. aeruginosa	1(.013)	-	-	-	1(.013)
Citrobacter spp	1(.013)	1(.013)	-	1(.013)	3(.04)
Acinetobacter spp	2(.026)	1(.013)	-	2(.026)	5(.067)
Enterobacter. Spp	3(.04)	-	-	1(.013)	4(.05)
S.arizona	1(.013)	-	-	-	1(.013)
p.vulgaris	1(.013)	-	-	1(.013)	2(.026)
B. pseudomallei	-	1(.013)	-	-	1(.013)
Klebsiella.spp	2(.026)	1(.013)	-	-	3(.04)
Providence	-	-	-	1(.013)	1(.013)
Subtotal Gram negative	**17(22.7%)**	**8(10.7%)**	**1(1.3%)**	**17(22.7%)**	**43(57.4%)**
Total	**38(50.7%)**	**18(.24%)**	**1(1.3%)**	**18(24%)**	**75(100%)**

P-value = 0.193

Antimicrobial susceptability pattern of bacterial isolates

Antimicrobial susceptibility testing was performed for all isolates according to the criteria of the CLSI by the disk diffusion method .Antibiotics-susceptibility pattern of gram posititve bacteria (N=32) against the tweelve antimicrobial agensts is shown on Table 5. The target bacteria *B. Pseudomallei* showed resisitance to ampicillin, ciprofloxacin, gentamicin and suscebtible to amoxicillin/clavulanic acid, ceftriaxone and ceftazidime.*Staphaylococcus aureus* showed high level of resisitance to Trimethoprim-sulphamethoxazole(65.5%),

Erythromycin(58.6%), Oxacillin (58.6%) and Gentamicin (55.2%). *Enterococcus spp* showedvery high (100%) resisitance to amoxicillin/clavulanic acid, Gentamicin, Trimethoprim-sulphamethoxazole, Ceftriaxone and Tetracycline. Both *S.aureus* and *Enterococcus spp* are highly suscebtible to Clindamycin (70%) and Vancomycin(100%) respectively(Table 6).

Overall, Klebsiella spp, *K.pneumoniae, Acinetobacter spp.* and *E. coli* showed resistance to most of the antibiotics. However, all gram negative isolates were found to be resisitant to Ampiciline .From the most resisitant isolates, *Acinetobacter spp.* was relatively less sensitive (60%) to the tested exposed antibiotics. Whereas, organisms such as *Klebsiella spp*. And *K.pneumoniae* showed 100% sentitive to Tetracycline, Imipenem and Amikacin, respectively. On the other hand, *E.coli* demonstrate a 100% resisitance to Tetracycline. The target organism, *B. Pseudomallei* showed 100% sensitivity to Amoxicillin-clavulanic acid,ceftriaxoneand Ceftazidime, intermidate to Trimethoprim-sulphamethoxazoleand resisitance to Gentamicin, Ciprofloxacin and ampicillin.

Table 5: Antibiotic susceptibility pattern of all isolatesof Gram-positive bacterial species

Bacterial isolates		**Antibiotics**											
		AMP	AUG	CIP	CN	SXT	CTR	ERY	OXA	TZP	CLI	TET	VAN
S.aureus(N=29)	s	5	7	13	13	7	8	10	12	ND	20	13	13
	I	10	10	4		3	13	2				6	5
	R	14	12	5	16	19	8	17	17		9	10	10
Enterococcus spp(N=3)	S	1		1				1	ND	1	ND		3
	I							1		1			
	R	2	3	2	3	3	3	1		1		3	
Total(N=32)	s												
	I												
	R												

AUG amoxicillin/clavulanic acid, SXT-trimethoprim-sulphamethoxazole, AMP-ampicillin, CIP-Ciprofloxacin,CN-gentamicin,CTR-ceftriaxone,ERY-Erythromycin,OXA-Oxacillin,TZPtazobactam,CLI-clindamycin,TET-Tetracycline,VAN-vancomycin,*ND-not done*

Table 6: Antibiotic susceptibility pattern of all isolates.of Gram Negative bacterial species

Bacterial isolates	**Antibiotics**														
		AMP	AUG	FOX	PEN	CIP	CN	SXT	CTR	CAZ	TZP	IPM	AMK	AMX	TET
K.peneumoniae(N=13)	s		3	3	3	4	4	2		3	6	13	13		6
	I		2			3									2
	R	13	8	10	10	6	9	11	13	10	7			7	5
E.coli(N=8)	s		1	ND	ND		3	7	3	2	6	ND	2	ND	
	I					1							4		
	R	8	7			7	5	1	5	6	2		2		8
Pseudomonas spp(N=1)	s							ND	ND	ND	ND	1	1	ND	ND
	I														
	R	1	1		1	1	1								
P. aeruginosa(N=1)	S			ND	ND			ND	ND			ND	1	ND	
	I														
	R	1	1			1	1			1	1				1
Citrobacter spp(N=3)	s	ND		ND		3	1					ND	ND	ND	1
	I														
	R		3				2	3	3	3	3				2
Acinetobacter spp(N=5)	s			ND	ND	2	3			2	ND		3		
	I											1			
	R	5	5			3	2	5	5	3		4	2	5	5
Enterobacter. Spp(N=4)	s		2	4	ND	3		ND			1	ND	ND	4	ND
	I					1					1				
	R	4	2				4		4	4	2				
S.arizona*(N=1)*	S			ND		1	ND	ND	ND	ND	ND	ND	ND	ND	1
	I		1												
	R	1													
p.vulgaris*(N=2)*	S	ND		ND	ND	1		ND			ND	ND	ND	ND	
	I		2												
	R					1	2		2	2					2
B. pseudomallei(N=1)	S		1	ND	ND				1	1	ND	ND	ND	ND	ND
	I							1							
	R	1				1	1								
Klebsiella.spp*(N=3)*	S				ND	1	1				1	ND	ND ND		3
	I		1												
	R	3	2	3		2	2	3	3	3	2				
Providence*(N=1)*	S	ND			ND	1	1	ND		ND					
	I														
	R		1	1					1						1
Total*(N=43)*	S														
	I														
	R														

*AMP-ampicillin, CIP- ciprofloxacin, CN- gentamicin, AUG- amoxicillin- clavulanic acid,FOX-Cefoxitin, PEN-Penicillin, SXT- trimethoprim-sulphamethoxazole, CAZ-ceftazidime, CTR-ceftriaxone,CAZ-ceftazidime,AMK-Amikacin,tzp-tazobactam,IPM-Imipenem,AMX-Amoxacilin, TET-Tetracycline**,** ND-not done*

6. DISCUSSION

Burkholderia pseudmallei considered as a potential emerging infectious disease in many tropical developing countries[20]. Ethiopia being in the tropics and with similar agroecological condition and soil type with other endemic areas we strongly suspects the bacteria may present and cause disease in the country. On the other hand reports from Steinmetz's laboratory (Germany) indicated that ananalysis of soil samples, taken from around Bahir Dar town and north region of Addis Ababain in November 2014 showed that about 50 % of the samples were found to be positive for *B. pseudomallei* based on multiple *B. pseudomallei*-specific qPCR test[24]. However, there is no officially recorded figure that show the extents and relevant of the disease in Ethiopia, which is probably due to the absence of bacteriological diagnostic resources[51], the over lapping of the clinical disease caused by *B. Pseudomallei* with other pyogenic infections[8] and a lack of awareness of the disease among clinicians. For instance,a report from Gambia, Mauritius,Madagascar,Malawi indicated that only one case report has been published sofar[35-37, 41].Such reports from other parts of the continent strength the hypothesis as well as the current finding of our study.

The current study shows that, relativly higher distribution 25% (7/28) of the bacterial strains were detected among age groups between 12-18 years followed by patients of >18 years old, 24% (49/206) and the least distribution was among younger patients aged <12 years old 23% (19/81)age group. Even though our result indicated that ageing has no association (P=0.986) with the occurrence of *B. pseudomallei* and othe bacterial infection. On the contrary, previous studies in Southeast Asiahave indicated a different result from our study, that is children with melioidosis are less likely to have localized disease (13%)[33, 34]. Even if our finding dipicts the absence of association between age and occurance of *B.Psuedominali* infection (p>0.05), but previous reports showed that the advancing age are commonly associated with risk factors including reduced immunity, co-morbid diseases such as diabetes mellitus, chronic heart diseases, neurogenic bladder [12]. Mean while other studies also showed that infants, lack of fully developed immunity, malnutrition as well as inadequate hygiene [52] might put them at a greater risk of infections.

The current study also indicated a higher distribution of bacterial infection among males (29%) than women (18%), with a p-value of 0.022^{*}). Our findings also showed that participants from rural areas (26%) are more affected by *B. pseudomallei* and other bacterial patogen than those from urban areas (22%), with a P-value of 0.322. Similarly, the rate of

exposure status per participants occupation indicated that the prevalence of *B. pseudomallei* and other bacterial patogen were higher (28%) among farmers than participants with different occupations (23%), with a P-value of 0.364(Table-2). This is probably due to the reason that the bacteria is commonly found in soil and then move to the surface with the rising water table during wet seasons[21, 44, 53]. Such condition may causes male patiens from rural area highly exposed to the *B.pseudomallei* infection.

Of the 315 clinical specimens received from Black Lion Hospital and Felege Hiwot hospital, 75(23.81%) isolates were found to be cultured positive. The highest rate of isolates were from pus, blood and urine, 41.86%, 21.71% and 20% respectively. A similar rates of isolates of *B.pseudomallei* (3.1 per 1000 clinical specemen) was reported by similar studies from Thiland [30]. These studies were reported from Thailand, to compare the isolation rates of *B.pseudomallei* among community-based hospitals located in the central, north, northeast, and south of Thailand from various clinical specimens during 1994-1995 and the result showed that the isolation rates of *B. speudomallei* were 4.2 and 4.1 per 1,000 clinical specimens in northeastern hospitals as compared to 1.1and 1.8 in central, 1.1 and 1.1 in north,and 1.2 and 0.7 per 1,000 clinical specimens in south Thailand[30]. Likewise, onother study from Thiland was also reported from Udon Thani Hospital, northeast Thailand, on 118 patients with suspected pulmonary tuberculosis indicated a relatively higher distribution (3/118) of *B. pseudomallei* in sputum culture[29].

The prevalence of isolated gram negative bacteria 43(57%) were higher in this study compared with similar studies in the country[46], but has almost similar finding with a study conducted in Gonder[47].*Staphylococcus.aurus, K.peneumoniae and E.coli* are the most prominent pathogens with the isolation rate of 29(39%), 13(17%) and 8(10%) respectively.This is consistent with other studies in Ethiopia(Gonder and Jimma)[47, 48].

Many reports are indicating *Melioidosis* is endemic in Southeast Asia and northern Australia, with the largest number of reports from Thailand[30-32]. This organism is found more commonly in cleared irrigated sites such as rice paddies and surface waters of endemic areas [5]. This report is strengthen our findings that shows an association of patients occupations with the occurrence of *B. pseudomallei* infection. That is, about 28%(64/315) of farmers were exposed to *B. pseudomallei* and other pathogens. we also get a 20 years age farmer with cutanious melodosis patient,on the other hand 72%(251/315) of patients from other field has got the infection from other pathogens (P=0.364). A study from Australia reported that,

B.pseudomallei has been found in clay soils at a depth of 25 to 45cms and it has been proposed that the bacteria move to the surface with the rising water table during wet seasons[44, 53]. The majority of melioidosis cases reported, occurred in monsoonal wet seasons[9, 43]. Melioidosis is increasingly recognized in southern India, and is an emerging infectious disease[7, 45, 54]. Similar other reports also indicated that, parts of Ethiopia were predicted to be environmentally suitable for *B. pseudomallei*. Such environmental studies revealed the presence of *B. pseudomallei* in soil samples in various regions of the country using molecular as well as cultural methods [24].

The identification of *B. pseudomallei* can be elusive, even in developed countries, as widely used automated identification systems, such as (Phoenix; BD Diagnostic Systems, Sparks, MD) or VITEK 2 [55]. Normally this bacterium confound with *B. cepacia*, a common respiratory pathogen commonly found in cystic fibrosis patients and lung nosocomial infections. The API 20 NE system is usually more accurate in the bacterial identification[56, 57] and in one study 792/800 (99%) *B.pseudomallei* isolates were correctly identified[48]. One clear problem during identification of *B. pseudomallei* from patient sample during routine clinical microbiological diagnosisis the lack of awareness of this disease in patients coming from non-endemic areas. In such cases, only a high index of clinical and microbiological suspicion can help in identifying such an isolate. For instance, the identification of a *Burkholderia spp*. with Gram negative, oxidase positive rod shap that has a colonial appearance typical of *B. pseudomallei* on Ashdown agar (also grow at 42°C) and is resistant to colistin and susceptible to amoxicillin/clavulanic acid, or a characteristic fenotype (wrinkled, lactosepositive colonies) of old cultures in Ashidwan agar can be identified as *B. Pseudomallei*[53, 56].

The result of antibiotic susceptibility testing based on the disk diffusion method, showed that*B. Pseudomallei*s demonstrated a 100% sensitivity to Amoxicillin- clavulanic acid,ceftriaxone and Ceftazidime, and an intermidate susptability to Trimethoprim-sulphamethoxazole; and resisitance to Gentamicin,Ampicillin and Ciprofloxacin. Isolates of *S.aurus* were most susceptible of gram positive bacteria to Clindamycin 69% (20/29) followed by Gentamicin 44.8 (13/29), Doxycycline 41.2% (12), Ceftriaxone 27.6% (8) and 24.1% (7) Trimethoprim-sulphamethoxazole. The most resistant drugs for *S.aurus* were Trimethoprim-sulphamethoxazole 65.5% (19/29) followed by 56.6% (17/29) Erythromycin,56.6% (17/29) Oxacillin, 55.2% (16/29) Gentamycin and 48.3(14/29)

Ampicillin. Similarly, all the gram negative isolates (12 isolates) were passed through the antibiotic susceptibility tests(Ampicillin, Ciprofloxacin, Gentamicin, Amoxicillin-clavulanic acid, Ceftriaxone, Doxycycline, Chloramphenicol, Norfloxacin, Trimethoprim-sulphamethoxazole, Ceftazidime, Cefuroxime, Streptomycin, Amikacin,Piperacillin-tazobactam, etc) and all of them exhibited resistance to most of the antibiotics, this is consistent with other studies in Ethiopia[46, 48].

Epidemiological surveillance of bacterial infection and resistance to antibiotics are essential for awareness creation, implementation of control measures and effective management of infections. This is important in developing countries particularly in sub-Saharan Africa where studies have indicated that many hospitals have rudimentary and poor enforcement of infection control measures and marginal awareness on the extent of infections caused by multi-drug resistant bacteria which have resulted in increased morbidity and mortality [58, 59].

7. Strengths and Limitations of the study

7.1.Strength of the study

- Since this study is the first study that isolate and report *Burkholderia pseudmallei from* clinical samples in Ethiopia, thus, it will provide important baseline information for future large-scale studies at national level.

7.2. Limitations of the study

- The target hospitals are far from those rice producer farmers. Because, *B. pseudominalei* estimated to be highly distributed in clay soil and among rice producing farmers due to their higher exposure status.

- At national level, the diagnostic methods of *B.Pseudominalei* is not well established as well as not incorporated in to the routine activities of the microbiology laboratories.

8. Conclusion and Recommendations

Overall, the current study has shown that the prevalence of bacterial pathoges is high. However, only one isolate of the target organism, *B. Pseudominalei* from clinical specimes was isolate from a pus sample of 20 years old farmers. This leads to the following recommendations.

1. Microbiology laboratories in the country need to be equipped and standard guidelines for the investigation of suspected melioidosis followed, considerning the environmental distribution of the target organism, it will become recognized as a major pathogen throughout the country.

2. Although developing an overall picture about melioidosis remaining an elusive undertaking, we strongly recommended that basic data about the actual figure of melioidosis identified from various speciments in variuose regions of the country is needed. To do so, nationwide surveillance should be undertaken with appropriate techniques.

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Annex 1: English version of the participant Information sheet andConsent form

Information sheet for Adult participants (> or =18 year)

Consent to participate in the study on profile and anti-microbial susceptibility of pathogenic bacteria from selected clinical specimens including *B. Pseudomallei.*

Name of Organization: Department of Medical Laboratory Science, Collage of Allied Health Sciences, Addis Ababa University, Addis Ababa, Ethiopia and Armauer Hansen Research Institute

Name of Sponsor: Armauer Hansen Research Institute

Title of the Research Project: Prevalence of *Burkholderia pseudomallei* and other bacterial pathogens in community acquired infections in different regions of Ethiopia.

Please read or listen when it is read for you about the general information of the study. If you have any question regarding the study please ask freely.

Background information

Respiratory, skin infection, sepsis and urinary tract infection causes great distress in terms of associated mortality and morbidity, increased length of hospital stay, profound discomfort and significant increase in healthcare cost. Therefore the knowledge of the causative agents of respiratory, skin infection, sepsis and urinary tract infection includingB.Pseudomalleiis which is the causative agent of melioidosis, a serious, often fatal disease of humanswill be helpful in the control and selection of empiric antimicrobial therapy as an infection control measure.

The reason of this study/Aim of the study

We are conducting research to find out bacterial cause of respiratory, skin infection, sepsis and urinary tract infection, including melioidosis,in this study area. This information will help treat patients with respiratory, skin infection, sepsis and urinary tract infection, in the future.

Melioidosisis a bacterial infection caused byB. Pseudomallei.The commonest routes ofB. Pseudomalleiinfection arethought inoculation, inhalation and ingestion.*It*is an environmental saprophyte found in wet soils. It mostly infects adults with an underlying predisposing condition, mainly diabetes mellitus. The disease can cause enormous clinical diversity,

spanning asymptomatic infection, localized skin ulcers orabscesses, chronic pneumonia mimicking tuberculosis, and fulminate septic shock with abscesses in multiple internal organs.

You have to do if youdecide to participate/Expected from participants

If you agree to take part you will be asked some questions. We will ask for your name, age, gender, ethnic group as well as your current illness, and whether you received any medication during the last week.

We will take isolated bacteriawhich is isolated during routine diagnosis for further bacteriology and genetic analyisis. As soon as the results of these tests are available you and/or your doctor will be informed.

Benefits

Study participants will not have any financial incentives or other inducements from participating on this study. If you participate in this research project, there may be direct or indirect benefit to you.Beside on the diagnosis result you will be treated accordingly. Most importantly, the result of the study will be beneficial to design effective prevention and control measure for respiratory, skin infection, sepsis and urinary tract infection. Hence, you are indirectly benefiting other patients and the society in this respect**.**

Risks /discomfort

There is no any risk in participating in this research project, If you agree to take part in this study, asking above mentioned questions may make feel uncomfortable. You may refuse answering any question or may take a break at any time. You may stop the participation in this study at any time.

Confidentiality

There is no sensitive issue that you will be asked related with your social desirability but all study records that identify you will be kept confidential. All information collected in this study will be given code numbers and no name will be recorded. The key to this code numbers will be kept in a locked file and be only accessible to authorized staff. Your name or any identifier will not be used in any publication or reports from this study. Results of investigations or other information that we collect about you will only be shared with medical staff looking after youand authorized members of the study such as officials from ethics committees

Right to refuse or withdraw

You have full right to refuse from participating in this research.You have also the full right to withdraw from this study at any time you wish, with out losing any of your right

You may refuse any physical examination, or answering any question or may take a break at any time.

You may stop the participation in this study at any time.

Principal investigator Address

About the conduct of the study, contact the following individual:

Emawayish Andarge, AHRI, Addis Ababa.

Phone number: +251911345215.

If you have any questions you may ask them now or later. If you wish to ask questions later, you may contact:

About AHRI / ALERT ethical clearance, contact the AAERC secretariat office, AHRI, Addis Ababa.Phone number: 0118-962183.

Informed Consent for Adult participants(> or =18 year)

The “Participant information sheet to participate in the Study on prevalence of *Burkholderia pseudomallei* and other bacterial pathogens in community acquired infections in addis ababa and bahir dar regions of ethiopia

The purpose and procedures, risks and benefits of this study have been explained to me in detail.

I have been allowed to ask questions, and my questions have been answered to my satisfaction by the research staff.

I have been told whom to contact if I have questions, want to discuss problems, or concerns.

I have been told that I will be given a signed and dated copy of this assent form.

I have been reassured that all information obtained as result of this study will be confidential and used for the purpose of this study only by the institutions participating in this study.

I assent voluntarily to my participation as a subject in this study. I will follow the directions of the study team and give them my full cooperation.

I understand that I have the right to withdraw from the study at any time, without any way affecting my own further medical care.

I the undersigned, have fully agreed to participate in the study"prevalence of *Burkholderia pseudomallei* and other bacterial pathogens in community acquired infections in addis ababa and bahir dar regions of ethiopia”

For Adult participant:

Name of participant: _____

Signature of participant: _____

Date: ____ / ____ / 20_____

If participant is illiterate:

Add name of independent literate witness (if possible, this person should be selected by the participant and should have no connection to the study team).

Name of witness: _____

Signature of witness: _____

Date: ____ / ____ / 20_____

For the study staff:

I have read/explained the study to the above named participant in a language that he/she understands well. I am certain that the participant has understood the information and is allowing 5-8 ml of blood/urine/pus/sputum to be taken out of his/her own free will.

Name of study staff: _____

Signature of study staff: _____

Date: ____ / ____ / 20_____

Information sheet for Adolescent participants(12-17 year)

Consent to participate in the Study on profile and anti-microbial susceptibility of pathogenic bacteria from selected clinical specimensincluding *B. Pseudomallei.*

Name of Organization: Department of Medical Laboratory Science, Collage of Allied Health Sciences, Addis Ababa University, Addis Ababa, Ethiopia and Armauer Hansen Research Institute

Name of Sponsor: Armauer Hansen Research Institute

Title of the Research Project: Prevalence of *Burkholderia pseudomallei* and other bacterial pathogens in community acquired infections in different regions of Ethiopia.

Please read or listen when it is read for you about the general information of the study. If you have any question regarding the study please ask freely.

Background information

Respiratory, skin infection, sepsis and urinary tract infection causes great distress in terms of associated mortality and morbidity, increased length of hospital stay, profound discomfort and significant increase in healthcare cost. Therefore the knowledge of the causative agents of respiratory, skin infection, sepsis and urinary tract infection includingB. Pseudomalleiis which is the causative agent of melioidosis, a serious, often fatal disease of humanswill be helpful in the control and selection of empiric antimicrobial therapy as an infection control measure.

The reason of this study/Aim of the study

We are conducting research to find out bacterial cause of respiratory, skin infection, sepsis and urinary tract infection, including melioidosis,in this study area. This information will help treat patients with respiratory, skin infection, sepsis and urinary tract infection, in the future.

Melioidosisis a bacterial infection caused by B. Pseudomallei.The commonest routes ofB. Pseudomallei infection arethought inoculation, inhalation and ingestion.*It*is an environmental saprophyte found in wet soils. It mostly infects adults with an underlying predisposing condition, mainly diabetes mellitus. The disease can cause enormous clinical diversity, spanning asymptomatic infection, localized skin ulcers orabscesses, chronic pneumonia mimicking tuberculosis, and fulminate septic shock with abscesses in multiple internal organs.

You have to do if you decide to participate/Expected from participants

If you agree to take part you will be asked some questions. We will ask for your name, age, gender, ethnic group as well as your current illness, and whether you received any medication during the last week.

We will take isolated bacteria which is isolated during routine diagnosis for further bacteriology and genetic analyisis. As soon as the results of these tests are available you and/or your doctor will be informed.

Benefits

Study participants will not have any financial incentives or other inducements from participating on this study. If you participate in this research project, there may be direct or indirect benefit to you.Beside on the diagnosis result you will be treated accordingly. Most importantly, the result of the study will be beneficial to design effective prevention and control measure for respiratory, skin infection, sepsis and urinary tract infection. Hence, you are indirectly benefiting other patients and the society in this respect**.**

Risks /discomfort

There is no any risk in participating in this research project, If you agree to take part in this study, asking above mentioned questions may make feel uncomfortable. You may refuse answering any question or may take a break at any time. You may stop the participation in this study at any time.

Confidentiality

There is no sensitive issue that you will be asked related with your social desirability but all study records that identify you will be kept confidential. All information collected in this study will be given code numbers and no name will be recorded. The key to this code numbers will be kept in a locked file and be only accessible to authorized staff. Your name or any identifier will not be used in any publication or reports from this study. Results of investigations or other information that we collect about you will only be shared with medical staff looking after youand authorized members of the study such as officials from ethics committees

Right to refuse or withdraw

You have full right to refuse from participating in this research.You have also the full right to withdraw from this study at any time you wish, with out losing any of your right

You may refuse any physical examination, taking blood or pus or answering any question or may take a break at any time.

You may stop the participation in this study at any time.

Principal investigator Address

About the conduct of the study, contact the following individual:

Emawayish Andarge, AHRI, Addis Ababa.

Phone number: +251911345215.

If you have any questions you may ask them now or later. If you wish to ask questions later, you may contact:

About AHRI / ALERT ethical clearance, contact the AAERC secretariat office, AHRI, Addis Ababa.Phone number: 0118-962183.

Informed Consent for Adolescent(12-17 year)

The "Participant information sheet to participate in the Study on prevalence of *Burkholderia pseudomallei* and other bacterial pathogens in community acquired infections in addis ababa and bahir dar regions of ethiopia

The purpose and procedures, risks and benefits of this study have been explained to me in detail.

My parents or guardian have to say to choose if I want to be in the study

I have been allowed to ask questions, and my questions have been answered to my satisfaction by the research staff.

I have been told whom to contact if I have questions, want to discuss problems, or concerns.

I have been told that I will be given a signed and dated copy of this assent form.

I have been reassured that all information obtained as result of this study will be confidential and used for the purpose of this study only by the institutions participating in this study.

I assent voluntarily to my participation as a subject in this study. I will follow the directions of the study team and give them my full cooperation.

I understand that I have the right to withdraw from the study at any time, without any way affecting my own further medical care.

I the undersigned, have fully agreed to participate in the study"prevalence of *Burkholderia pseudomallei* and other bacterial pathogens in community acquired infections in addis ababa and bahir dar regions of ethiopia"

For adolescentparticipant's:

Name of participant: _____

Signature of participant: _____

Date: ____ / ____ / 20_____

If participant is illiterate:

Add name of independent literate witness (if possible, this person should be selected by the participant and should have no connection to the study team).

Name of witness: _____

Signature of witness: _____

Date: ____ / ____ / 20_____

For the study staff:

I have read/explained the study to the above named participant in a language that he/she understands well. I am certain that the participant has understood the information and is allowing 5-8 mlof blood/urine/pus/sputum to be taken out of his/her own free will.

Name of study staff: _____

Signature of study staff: _____

Date: ____ / ____ / 20_____

Information sheet for parent/guardianin (case of participants with age of < 18yrs)

Consent for your child participation in the Study on profile and anti-microbial susceptibility of pathogenic bacteria from selected clinical specimensincluding *B. Pseudomallei*

Name of Organization: Department of Medical Laboratory Science, Collage of Allied Health Sciences, Addis Ababa University, Addis Ababa, Ethiopia and Armauer Hansen Research Institute

Name of Sponsor: Armauer Hansen Research Institute

Title of the Research Project: Prevalence of *Burkholderia pseudomallei* and other bacterial pathogens in community acquired infections in different regions of Ethiopia

Please read or listen when it is read for you about the general information of the study. If you have any question regarding the study please ask freely.

Background information

Background: Respiratory, skin infection, sepsis and urinary tract infection causes great distress in terms of associated mortality and morbidity, increased length of hospital stay, profound discomfort and significant increase in healthcare cost. Therefore the knowledge of the causative agents of respiratory, skin infection, sepsis and urinary tract infection including B. pseudomalleiis which is the causative agent of melioidosis, a serious, often fatal disease of humans will be helpful in the control and selection of empiric antimicrobial therapy as an infection control measure.

The reason of this study/Aim of the study

We are conducting research to find out bacterial cause of respiratory, skin infection, sepsis and urinary tract infection, including melioidosis,in this study area. This information will help treat patients with respiratory, skin infection, sepsis and urinary tract infection, in the future.Melioidosis, is a bacterial infection caused byB. Pseudomallei.The commonest routes ofB. Pseudomalleiinfection arethought to be inoculation, inhalation and ingestion.It is an environmental saprophyte found in wet soils. It mostly infects adults with an underlying predisposing condition, mainly diabetes mellitus. The disease can cause enormous clinical diversity, spanning asymptomatic infection, localized skin ulcers orabscesses, chronic pneumonia mimicking tuberculosis, and fulminate septic shock with abscesses in multiple internal organs.

Your child has to do if you decide for his/her participation/ Expected from participants

If you agree for your child's participation we will ask some questions about your child. These include his/her name, age, gender, ethnic group as well as his/her current illness, and whether he/she received any medication during the last week.
We will take isolated bacteria which is isolated during routine diagnosis for further bacteriology and genetic analyisis. As soon as the results of these tests are available you and/or your doctor will be informed.

Benefits

Study participants will not have any financial incentives or other inducements from participating on this study. If your child participates in this research project, there may be direct or indirect benefit to your child.Beside on the diagnosis result your child will be treated accordingly. Most importantly, the result of the study will be beneficial to design effective prevention and control measure for respiratory, skin infection, sepsis and urinary tract infection. Hence, you/ your child are indirectly benefiting other patients and the society in this respect.

Risks /discomfort

There is no any risk in participating in this research project,If you agree to take partyour child in this study, asking above mentioned questions may make feel uncomfortable. You/yourchild may refuse answering any question or may take a break at any time. You may stop the participation of your daughter's/son's in this study at any time.

Confidentiality

There is no sensitive issue that you will be asked related with you/your child social desirability but all study records that identify your child will be kept confidential. All information collected in this study will be given code numbers and no name will be recorded. The key to this code numbers will be kept in a locked file and be only accessible to authorized staff. Your child name or any identifier will not be used in any publication or reports from this study. Results of investigations or other information that we collect about your child will only be shared with medical staff looking after youand authorized members of the study such as officials from ethics committees.

Right to refuse or withdraw

You/your child have full right to refuse from participating in this research. You/your child have also the full right to withdraw from this study at any time you wish, with out losing any of your right

You/your child may refuse any physical examination, taking blood or pus or answering any question or may take a break at any time.

You/your child may stop the participation in this study at any time.

Principal investigator Address

About the conduct of the study, contact the following individual:

Emawayish Andarge, AHRI, Addis Ababa.

Phone number: +251911345215.

If you have any questions you may ask them now or later. If you wish to ask questions later, you may contact:

About AHRI / ALERT ethical clearance, contact the AAERC secretariat office, AHRI, Addis Ababa.Phone number: 0118-962183.

Informed Consent from parent/guardian

The "Consent to my child's participation in the Study on profile and anti-microbial susceptibility of pathogenic bacteria from selected clinical specimensincluding *B. Pseudomallei* has been read to me/ I have read.

The purpose and procedures, risks and benefits of this study have been explained to me in detail.

I have been allowed to ask questions, and my questions have been answered to my satisfaction by the research staff.

I have been told whom to contact if I have questions, want to discuss problems, or concerns.

I have been told that I will be given a signed and dated copy of this assent form.

I have been reassured that all information obtained as result of this study will be confidential and used for the purpose of this study only by the institutions participating in this study.

I assent voluntarily to my participation as a subject in this study. I will follow the directions of the study team and give them my full cooperation.

I understand that I have the right to withdraw my child from the study at any time, without any way affecting my child further medical care.

I the undersigned, have fully agreed to participate in the study"prevalence of *Burkholderia pseudomallei* and other bacterial pathogens in community acquired infections in addis ababa and bahir dar regions of ethiopia"

Name of participant child/adolescent:_____

For Parent/guardian:

Name of parent/guardian: _____

Signature/thumbprint of participant or parent/guardian: _____

Date: ____ / ____ / 20_____

If parent/guardian is illiterate:

Add name of independent literate witness (if possible, this person should be selected by the parent/guardian and should have no connection to the study team).

Name of witness: _____

Signature of witness: _____

Date: ____ / ____ / 20_____

For the study staff:

I have read/explained the study to the above named - parent/guardian in a language that he/she understands well. I am certain that the parent/guardian has understood the information and is allowing 2-4 mlof blood/urine/pus/sputum to be taken out of his/her own free will.

Name of study staff: _____

Signature of study staff: _____

Date: _____ / ____ / 20____

Annex2.Amharic Version of the participant Information sheetand Consent form

<u>በማወቅ የሚሰጥ የፈቃደኝነት ቅፅ ለአዋቂ የጥናት ተሳታፊ(18 አመት ና ከዚያ በላይ)</u>

ከተመረጡየምርመራ ናሙና ውስጥ በሽታ በሚያመጡ የባክቴሪያ አይነቶችን መለየት፤የፀረ ባክቴሪያመዳኒትየመቋቋምባህሪያቸውን ማወቅ፤ ብሩክሆልድሪያ ሲዶማሊ የተባለውን ባክቴሪያንጨምሮ የዳሰሳ ጥናትለሚሳተፉየተዘጋጀ **ቅፅ**

የድርጅቱ ስም፡-አዲስአበባዩኒቨርሲቲየጤናሳይንስኮሌጅየህክምናላብራቶሪሳይንስዲፓርትመንት እናአርማወር ሀንሰን የምርምር ተቋም

የስፖንሰሩ ስም ፡- አርማወር ሀንሰን የምርምር ተቋም

አርስት

በሚካሄደው ከተመረጡ የምርመራ ናሙና ውስጥ በሽታ በሚያመጡ የባክቴሪያ አይነቶችን መለየት፤ የፀረ ባክቴሪያመዳኒትየመቋቋምባህሪያቸውን ማወቅ፤ ብሩክሆልድሪያ ሲዶማሊ የተባለውን ባክቴሪያ በተሻሻለ የባክቴሪያ ማሳደግ እና ከአፈር የዳሰሳ ጥናት።

አጠቃላይ መረጃ

በጥናቱለመሳተፍከመወሰንዎበፊትይህንቅፅበትክክልአንብቡወይምሲነበብልዎበትክክልያዳምጡ፤ እንዲሁምግልጽያልሆነልዎትንነገርበሙሉነፃነትm ይጠይቁ።

ስለጥናቱመረጃ

የሳንባ ምች፣ የደም መቆሸሸሽ፣ የቆዳ መመርቀዝ እናየሽንት ቧንቧመመርቀዝሀመሞች በህመምተኛውላይየተለያዩችግሮችን ሊያስከትሉ ይችላሉ።ለምሳሌየህክምናወጭንይጨምራል፣ ለህመምተኛውምቾትይነሳል፣ሆስፒታልውስጥ ተኝቶየመታከምጊዜንያራዝማልብሎምለሞትሊዳርግይችላል።ስለዚህምየሳንባ ምች፣ የደም መቆሸሸሽ፣ የቆዳ መመርቀዝ እናየሽንት ቧንቧ መመርቀዝህመምሊያመጡየሚችሉባክቴሪያዎችብሩክሆልድሪያ ሲዶማሊን (ሜሎዶሲስ የሚባል በሽታ አምጭ ባክቴሪያን) ጨምሮ የበሽታውን ምንጭ ማወቅህመሙንለመቆጣጠርናለመከላከልይጠቅማል።

የጥናቱ ዓላማ

የዚህ ጥናት ዓላማ ሜሎዶሲስን ጨምሮ የሳንባ ምች፣ የደም መቆሸሸሽ፣ የቆዳ መመርቀዝ እናየሽንት ቧንቧ መመርቀዝ የሚያመጡ በሽታዎችን በአዲስ አበባ እና በባህር ዳር እንዲሁምአካባቢያቸው ምን ያህል እንደሚታዩ ማወቅ ነው። ከጥናቱ የሚገኘው መረጃ ለወደፊት ሜሎዶሲስን ጨምሮ የሳንባ ምች፣ የደም መቆሸሸሽ፣ የቆዳ መመርቀዝ እናየሽንት ቧንቧ መመርቀዝ የሚያስከትሉ በሽታዎችን ለማከምና ለመቆጣጠር ይረዳል።

ሜሎዶሲስብሩክሆልድሪያ ሲዶማሊን በሚባል ባክቴሪያ አማካኝነት የሚመጣ በሽታ ሲሆን በዋናነት የሚተላለፈው በንክኪ፣ በአየርእና በተበከለ ምግብአማካኝነት ነው። በሽታው ከቀላል እስከ ከባድ ህመም የሚያደርስ ሲሆን ብሎም ለሞት ሊዳርግ ይችላል።

በጥናቱ ለመካተት ከወሰኑ መደረግ ያለበት

በጥናቱ ለመካተት ከወሰንክ/ሽ አንዳንድ ጥያቄዎች እንጠይቅሃ/ሻለን። ከጥያቄዎቹ መካከል፤ ስም፤ ዕድሜ፤ ጾታ፤ ብሄርና ስለህመምህ/ሽ ሁኔታ ይገኙበታል። በተጨማሪም በሽታውን ለማስታገስ ምናልባት የወሰድካቸው/ሻቸው መድሃኒቶች ስለመኖራቸው ትጠየቃለህ/ሽ። እንዲሁምለምርመራ ከሰጡት ናሙና በሽታ ያመጣው ባክቴሪያ ለተጨማሪ የባክቴሪዮሎጂ እና የዘረመል ምርመራ እንወስዳለን፤ ውጤቱም እንደደረሰ የሚገለፅ ይሆናል። ምርመራውም ስም በሌለው በተለዬ ኮድ ይካሄዳል።

ለጥናቱ ተሳታፊዎች ላይ ያለው ጥቅም

በጥናቱ ለሚሳተፉ ፍቃደኛ ተሳታፊዎች ምንም አይነት የገንዘብ ክፍያ የለም። ነገር ግን በምርመራው ውጤት መሰረት የመታከም እድል ይኖሮታል።በተጨማሪም የጥናቱ ውጤት የሳንባ ምች፣ የደም መቆሸሸሽ፣ የቆዳ መመርቀዝ እና የሽንት ቧንቧመመርቀዝ ለመቆጣጠርናለመከላከል ስለሚጠቅም በተዘዋዋሪ መንገድ ሌላ ህመምተኛ እንዲሁምህብረተሰቡን የመጥቀም እድልያገኛሉ።

በጥናቱተሳታፊዎችላይያለውጉዳት

ደምበሚወሰድበትጊዜበመርፌምክንያትየሚከሰትመጠነኛህመምወይምመበለዝሊኖርይችላል።

ይህምከጥቂትጊዜበኋላየሚጠፋሲሆንሌላይህነውየሚባልጉዳትአይኖርም። ናሙና ከቁስል ላይ ሲወሰድ ከመጠነኛ ስሜት በስተቀር በጤናዎ ላይ ምንም ጉዳት አይደርስም።ሽንትና አክታሲወሰድበጤናዎ ላይ

ምንም ጉዳት አይደርስም።ምስቲራዊነትያላቸውመረጃዎችስለሚኖሩስጋትሊያድርብህ/ሽይችላል። ሆኖምየእነዚህመረጃዎችምስጢራዊነትበሚገባየተጠበቀነው።5-8 ሚሊ ሊትርየደም፡ጥቂትየሽንት፤የአክታ ወይምየቁስልመውሰድእንዲሁምከላይየተገለፁትንምርመራዎችናመጠይቆችማድረግምናልባትመጠነኛየም ቾትመጓደልሊያስከትልብህ/ሽይችላል።

ጥያቄዎችንአለመመለስምርመራዎቹንናደምመውሰዱንምእንቢማለትወይምየእረፍትጊዜመውሰድትችላለህ /ሽ።በፈለግህ/ሽበትጊዜምከነጭራሹራስህ/ሽንከጥናቱማግለልትችላለህ/ያለሽ::

የመረጃሚስጥራዊአጠባበቅ

አንተ/ችንሊለይየሚያስችልመረጃበሙሉበሚስጥርይያዛል።

በዚህጥናትየሚሰበሰበውመረጃበሙሉየኮድቁጥርየሚሰጠውሲሆንምንምዓይነትስምአይመዘገብም:: የዚህኮድመፍቻበፋይልተቆልፎየሚቀመጥሲሆንየተፈቀደለትሰውብቻፋይሉንማየትይችላል። ከዚህጥናትበሚወጡዘገባዎችወይንምየህትመትውጤቶችላይስምንወይምሌላአንተ/ችንሊያስለይየሚያ ስችልመረጃአይኖርም።

ከምርመራየሚገኝውምውጤትወይምሌላመረጃለሚመለከታቸውለምሳሌአንተ/ችንየሚንከባከቡየህክ ምናባለሙዎችእናጥናቱንለሚያካሄዱትባለሙያዎችእንዲሁምጥናቱስነምግባርንጠብቆመከናወኑንለ ሚከተሉየመንግስትአካለትብቻይገለፃል።

ጥናቱን እንቢ ማለት ወይንም ቋረጥ መብት

በዚህ ጥናት ያለመሳተፍ ወይንም ከጥናቱ አቋርጦ የመውጣት ሙሉ መብት አለወት።

ጥያቄዎችን አለመመለስ ምርመራዎቹንና ደም መውሰዱንም እንቢ ማለት ወይም የእረፍት ጊዜ መውሰድ ትችላለህ/ሽ።በፈለግህ/ሽበት ጊዜም ከነጭራሹ ራስህ/ሽን ከጥናቱ ማግለል ትችላለህ/ያለሽ::

ማንኛውም ዓይነት ጥያቄ ቢኖርዎት

ማንኛውም ዓይነት ጥያቄ ቢኖርዎት አሁን ወይም በማኝኛውም ጊዜ መጠየቅ ይችላሉ። በሌላ ጊዜ ጥያቄ ቢኖርዎት በዚህ አድራሻ ሊያገኙን ይችላሉ።

እማዋይሽ አንዳርጌ፤በስልክ ቁጥር 0911345215

እና አለርት/አህሪ ኢቲክስ ኮሚቴ 0118-962183

<u>የአዋቂ የጥናት ተሳታፊ የሚሰጥየፈቃደኝነትቃል</u>

- ከተመረጡ የምርመራ ናሙና ውስጥ በሽታ በሚያመጡ የባክቴሪያ አይነቶችን መለየ፤የፀረ ባክቴሪያመዳኒትየመቋቋምባህሪያቸውን ማወቅ፤ ብሩክሆልድሪያ ሲዶማሊ የተባለውን ባክቴሪያጨምሮየዳሰሳ ጥናት ለሚሳተፉ ከተዘጋጀ ቅፅየፈቃደኝነት ቅፅን አንብቤያለሁ ወይምን ተነቦልኛል።
- ዓላማውና የአሰራሩ ሁኔታ&የጥናቱ ጥቅሞችና ሊኖሩ የሚችሉ ስጋቶች በሙሉ በተሟላ ሁኔታ ተገልፆልኛል።
- ጥያቄዎችን እንድጠይቅ ተፈቅዶልኛል እንዲሁም ለጥያቄዎቹ በጥናቱ ባለሙያዎች አጥጋቢ መልስ አግኝቻለሁ። ጥያቄ ቢኖረኝ ወይም የከነከኑኝን ነገሮች ካሉ ማንን ማናገር እንደምችል ተነግሮኛል::
- የተፈረመና ቀን የተፃፈበት ይህ የፈቃደኝነት ቅፅ እንደሚሰጠኝና በጥናቱ የሚገኙ መረጃዎች ሚስጥራዊነት እንደሚጠበቅና ከጥናቱ ዓላማ ውጪ እንደማይውል እንዲሁም ጥናቱን ከሚያካሂዱት ተቋማት ውጪ እንደማይወጣ መተማመኛ ተሰጥቶኛል::
- ስለሆነም በዚህ ጥናት ውስጥ ለመሳተፍ ፈቅጃለሁ። የጥናቱ ባለሙዎች የሚሰጡኝን መመሪያ እከተላለሁ እንዲሁም ሙሉ በሙሉ ከእነርሱ ጋር እተባበራለሁ።
- በማናቸውም ጊዜ ከጥናቱ ራሴን ማግለል እንደምችልና ይህም የሚደረግልኝን ህክምና እንደማይለውጥ አውቄያለሁ። በዚህ ጥናት ባለመሳተፌ የሚኖር ቅጣት ወይም የሚጓደል ጥቅም አለመኖሩን አውቃለሁ።
- እኔ “ከተመረጡ የምርመራ ናሙና ውስጥ በሽታ በሚያመጡ የባክቴሪያ አይነቶችን መለየ፤ የፀረ ባክቴሪያ መዳኒት የመቋቋም ባህሪያቸውን ማወቅ፤ ብሩክሆልድሪያ ሲዶማሊ የተባለውን ባክቴሪያ ጨምሮ ዳሰሳ”ጥናትለመሳተፍ ተስማምቸ ከታች ፈርሜለሁ

ለተሳታፊ

- የተሳታፊ ስም ፡- _____
- የተሳታፊ ፊርማ/የጣት አሻራ _____ ቀን፤ ----------

ለምስክር

- ተሳታፊ/ዋ ማንብብና መፃፍ የማይችሉ ከሆነ ማንሀብና መፃፍ የሚችል ገለልተኛ ምስክር፤ (ቢቻል ምስክሩ በተሳታፊው/ዋ ቢጠቆሙ ይመረጣል። በተጨማሪም ከጥናቱ ባለሙዎች ጋር ግንኙነት ሊኖራቸው አይገባም)

- የምስክሩ ስም፤ ----------
- የምስክሩ ፊርማ፤ ---------- ቀን፤ ----------

ለተመራማሪ/ለሐኪም

- ተሳታፊው/ዋ በዚህ ጥናት ለመሳተፍ ከመወሰኑ/ኗ በፊት በቂ መረጃና ማብራሪያ በሚገባው/ት ቋንቋና መንገድ እንዲያገኝ/እንድታገኝ አድርጊያለሁ። ስለሆነም ተሳታፊው/ዋ ጉዳዩን በሚገባ እንደተረዳ/ችው እርግጠኛ ነኝ፤ 5-8 ሚሊ ሊትርየደም፡ጥቂትየሽንት፤የአክታ ወይም የቁስል ናሙናም እንድንወስድ ፈቅዷል/ፈቅዳለች።
- የተመራማሪው ስም፤ ----------
- የተመራማሪው ፊርማ፤ ---------- ቀን፤ ----------

<u>በማወቅ የሚሰጥ የፈቃደኝነት ቅፅ ለታዳጊ ወጣት የጥናት ተሳታፊ(ከ 12-17 አመት)</u>

ከተመረጡየምርመራ ናሙና ውስጥ በሽታ በሚያመጡ የባክቴሪያ አይነቶችን መለየት፤የፀረ ባክቴሪያመዳኒትየመቋቋምባህሪያቸውን ማወቅ፤ ብሩክሆልድሪያ ሲዶማሊ የተባለውን ባክቴሪያንጨምሮ የዳሰሳ ጥናትለሚሳተፉየተዘጋጀ ቅፅ

የድርጅቱ ስም፡-አዲስአበባዩኒቨርሲቲየጤናሳይንስኮሌጅየህክምናላብራቶሪሳይንስዲፓርትመንት እናአርማወር ሀንሰን የምርምር ተቋም

የስፖንሰሩ ስም ፡- አርማወር ሀንሰን የምርምር ተቋም

አርስት

በሚካሄደው ከተመረጡ የምርመራ ናሙና ውስጥ በሽታ በሚያመጡ የባክቴሪያ አይነቶችን መለየት፤ የፀረ ባክቴሪያመዳኒትየመቋቋምባህሪያቸውን ማወቅ፤ ብሩክሆልድሪያ ሲዶማሊ የተባለውን ባክቴሪያ በተሻሻለ የባክቴሪያ ማሳደግ እና ከአፈር የዳሰሳ ጥናት።

አጠቃላይ መረጃ

በጥናቱለመሳተፍ ከመወሰንዎበፊትይህንቅፅበትክክልአንብቡወይምሲነበብልዎበትክክልያዳምጡ፤ እንዲሁምግልጽያልሆነልዎትንነገርበሙሉነፃነትm ይጠይቁ።

ስለጥናቱመረጃ

የሳንባ ምች፣ የደም መቆሸሸሸ፣ የቆዳ ኢንፌክሽን እናየሽንት ቧንቧኢንፌክሽንህመሞች በህመምተኛውላይየተለያዩችግሮችን ሊያስከትሉ ይችላሉ።ለምሳሌየህክምናወጭንይጨምራል፣ ለህመምተኛውምቾትይነሳል፣ሆስፒታልውስጥ

ተኝቶየመታከምጊዜንያራዝማልብሎምለሞትሊዳርግይችላል።ስለዚህምየሳንባ ምች፣ የደም መቆሸሸሸ፣ የቆዳ መመርቀዝ እናየሽንት ቧንቧ መመርቀዝ ህመምሊያመጡየሚችሉባክቴሪያዎችብሩክሆልድሪያ ሲዶማሊን (ሜሎዶሲስ የሚባል በሽታ አምጭ ባክቴሪያን) ጨምሮ የበሽታውን ምንጭ ማወቅህመሙንለመቆጣጠርናለመከላከልይጠቅማል።

የጥናቱ ዓላማ

የዚህ ጥናት ዓላማ ሜሎዶሲስን ጨምሮ የሳንባ ምች፣ የደም መቆሸሸሸ፣ የቆዳ መመርቀዝ እናየሽንት ቧንቧ መመርቀዝ የሚያመጡ በሽታዎችን በአዲስ አበባ እና በባህር ዳር እንዲሁምአካባቢያቸው ምን

ያህል እንደሚታዩ ማወቅ ነው። ከጥናቱ የሚገኘው መረጃ ለወደፊት ሜሎዶሲስን ጨምሮ የሳንባ ምች፣ የደም መቆሸሸሸ፣ የቆዳ መመርቀዝ እናየሽንት ቧንቧ መመርቀዝ የሚያስከትሉ በሽታዎችን ለማከምና ለመቆጣጠር ይረዳል።

ሜሎዶሲስብሩክሆልድሪያ ሲዶማሊን በሚባል ባክቴሪያ አማካኝነት የሚመጣ በሽታ ሲሆን በዋናነት የሚተላለፈው በንክኪ፣ በአየርእና በተበከለ ምግብአማካኝነት ነው። በሽታው ከቀላል እስከ ከባድ ህመም የሚያደርስ ሲሆን ብሎም ለሞት ሊዳርግ ይችላል።

በጥናቱ ለመካተት ከወሰኑ መደረግ ያለበት

በጥናቱ ለመካተት ከወሰንክ/ሽ አንዳንድ ጥያቄዎች እንጠይቅሃ/ሻለን። ከጥያቄዎቹ መካከል፤ ስም፤ ዕድሜ፤ ጾታ፤ ብሄርና ስለህመምህ/ሽ ሁኔታ ይገኙበታል። በተጨማሪም በሽታውን ለማስታገስ ምናልባት የወሰድካቸው/ሻቸው መድሃኒቶች ስለመኖራቸው ትጠየቃለህ/ሽ። እንዲሁምለምርመራ ከሰጡት ናሙና በሽታ ያመጣው ባክቴሪያ ለተጨማሪ የባክቴሪዮሎጂ እና የዘረመል ምርመራ እንወስዳለን፤ ውጤቱም እንደደረሰ የሚገለፅ ይሆናል። ምርመራውም ስም በሌለው በተለዬ ኮድ ይካሄዳል።

ለጥናቱ ተሳታፊዎች ላይ ያለው ጥቅም

በጥናቱ ለሚሳተፉ ፍቃደኛ ተሳታፊዎች ምንም አይነት የገንዘብ ክፍያ የለም። ነገር ግን በምርመራው ውጤት መሰረት የመታከም እድል ይኖሮታል።በተጨማሪም የጥናቱ ውጤት የሳንባ ምች፣ የደም መቆሸሸሸ፣ የቆዳ መመርቀዝ እና የሽንት ቧንቧመመርቀዝ ለመቆጣጠርናለመከላከል ስለሚጠቅም በተዘዋዋሪ መንገድ ሌላ ህመምተኛ እንዲሁምህብረተሰቡን የመጥቀም እድልያገኛሉ።

በጥናቱተሳታፊዎችላይያለውጉዳት

ደምበሚወሰድበትጊዜበመርፌምክንያትየሚከሰትመጠነኛህመምወይምመበለዝሊኖርይችላል።

ይህምከጥቂትጊዜበኋላየሚጠፋሲሆንሌላይህነውየሚባልጉዳትአይኖርም። ናሙና ከቁስል ላይ ሲወሰድ ከመጠነኛ ስሜት በስተቀር በጤናዎ ላይ ምንም ጉዳት አይደርስም።ሽንትና አክታሲወሰድበጤናዎ ላይ ምንም ጉዳት አይደርስም።ምስቲራዊነት ያላቸው መረጃዎች ስለሚኖሩ ስጋት ሊያድርብህ/ሽይችላል። ሆኖም የእነዚህ መረጃዎች ምስጢራዊነት በሚገባ የተጠበቀ ነው። 5-8 ሚሊ ሊትርየደም፡ጥቂትየሽንት፤ የአክታ ወይም የቁስል መውሰድ እንዲሁም ከላይ የተገለፁትን ምርመራዎችና መጠይቆች ማድረግ ምናልባት መጠነኛ የምቾት መጓደል ሊያስከትልብህ/ሽይችላል።ጥያቄዎችን አለመመለስ ምርመራዎቹንና

ደም መውሰዱንም እንቢ ማለት ወይም የእረፍት ጊዜ መውሰድ ትችላለህ/ሽ።በፈለግህ/ሽበት ጊዜም ከነጭራሹ ራስህ/ሽን ከጥናቱ ማግለል ትችላለህ/ያለሽ::

የመረጃሚስጥራዊአጠባበቅ

አንተ/ችንሊለይየሚያስችልመረጃበሙሉበሚስጥርይያዛል።

በዚህጥናትየሚሰበሰበውመረጃበሙሉየኮድቁጥርየሚሰጠውሲሆንምንምዓይነትስምአይመዘገብም::

የዚህኮድመፍቻበፋይልተቆልፎየሚቀመጥሲሆንየተፈቀደለትሰውብቻፋይሉንማየትይችላል።

ከዚህጥናትበሚወጡዘገባዎችወይንምየህትመትውጤቶችላይስምንወይምሌላአንተ/ችንሊያስለይየሚያስችልመረጃአይኖርም።

ከምርመራየሚገኘውምውጤትወይምሌላመረጃለሚመለከታቸውለምሳሌአንተ/ችንየሚንከባከቡየህክምናባለሙዎችእናጥናቱንለሚያካሄዱትባለሙያዎችእንዲሁምጥናቱስነምግባርንጠብቆመከናወኑንለሚከተሉየመንግስትአካለትብቻይገለፃል።

ጥናቱን እንቢ ማለት ወይንም ቋረጥ መብት

በዚህ ጥናት ያለመሳተፍ ወይንም ከጥናቱ አቋርጦ የመውጣት ሙሉ መብት አለወት።

ጥያቄዎችን አለመመለስ ምርመራዎቹንና ደም መውሰዱንም እንቢ ማለት ወይም የእረፍት ጊዜ መውሰድ ትችላለህ/ሽ።በፈለግህ/ሽበት ጊዜም ከነጭራሹ ራስህ/ሽን ከጥናቱ ማግለል ትችላለህ/ያለሽ::

ማንኛውም ዓይነት ጥያቄ ቢኖርዎት

ማንኛውም ዓይነት ጥያቄ ቢኖርዎት አሁን ወይም በማኝኛውም ጊዜ መጠየቅ ይችላሉ። በሌላ ጊዜ ጥያቄ ቢኖርዎት በዚህ አድራሻ ሊያገኙን ይችላሉ።

እማዋይሽ አንዳርጌ፤በስልክ ቁጥር 0911345215 እና አለርት/አህሪ ኢቲክስ ኮሚቴ 0118-962183

ለታዳጊ ወጣት የጥናት ተሳታፊ የሚሰጥየፈቃደኝነትቃል

- ከተመረጡ የምርመራ ናሙና ውስጥ በሽታ በሚያመጡ የባክቴሪያ አይነቶችን መለየ፤የፀረ ባክቴሪያመዳኒትየመቋቋምባህሪያቸውን ማወቅ፤ ብሩክሆልድሪያ ሲዶማሊ የተባለውን ባክቴሪያጨምሮየዳሰሳ ጥናት ለሚሳተፉ ከተዘጋጀ ቅፅየፈቃደኝነት ቅፅን አንብቤያለሁ ወይምን ተነቦልኛል።
- ዓላማውና የአሰራሩ ሁኔታ፤የጥናቱ ጥቅሞችና ሊኖሩ የሚችሉ ስጋቶች በሙሉ በተሟላ ሁኔታ ተገልፆልኛል።

- ወላጆቼም/ አሳዳጊዎቼምበጥናቱእንድሳተፍ ወይም እንዳልሳተፍምርጫውየእኔመሆኑንነግረውኛል።
- ጥያቄዎችን እንድጠይቅ ተፈቅዶልኛል እንዲሁም ለጥያቄዎቹ በጥናቱ ባለሙያዎች አጥጋቢ መልስ አግኝቻለሁ። ጥያቄ ቢኖረኝ ወይም የከነከኑኝን ነገሮች ካሉ ማንን ማናገር እንደምችል ተነግሮኛል::
- የተፈረመና ቀን የተፃፈበት ይህ የፈቃደኝነት ቅፅ እንደሚሰጠኝና በጥናቱ የሚገኙ መረጃዎች ሚስጥራዊነት እንደሚጠበቅና ከጥናቱ ዓላማ ውጪ እንደማይውል እንዲሁም ጥናቱን ከሚያካሂዱት ተቋማት ውጪ እንደማይወጣ መተማመኛ ተሰጥቶኛል::
- ስለሆነም በዚህ ጥናት ውስጥ ለመሳተፍ ፈቅጃለሁ። የጥናቱ ባለሙዎች የሚሰጡኝን መመሪያ እከተላለሁ እንዲሁም ሙሉ በሙሉ ከእነርሱ ጋር እተባበራለሁ።
- በማናቸውም ጊዜ ከጥናቱ ራሴን ማግለል እንደምችልና ይህም የሚደረግልኝን ህክምና እንደማይለውጥ አውቄያለሁ። በዚህ ጥናት ባለመሳተፌ የሚኖር ቅጣት ወይም የሚጓደል ጥቅም አለመኖሩን አውቃለሁ።
- እኔ “ከተመረጡ የምርመራ ናሙና ውስጥ በሽታ በሚያመጡ የባክቴሪያ አይነቶችን መለየ፤ የፀረ ባክቴሪያ መዳኒት የመቋቋም ባህሪያቸውን ማወቅ፤ ብሩክሆልድሪያ ሲዶማሊ የተባለውን ባክቴሪያ ጨምሮ ዳሰሳ”ጥናትለመሳተፍ ተስማምቸ ከታች ፈርሜለሁ

ለተሳታፊ

- የተሳታፊ ስም ፡- _____
- የተሳታፊ ፊርማ/የጣት አሻራ _____ ቀን፤ ----------

ለምስክር

- ተሳታፊ/ዋ ማንብብና መፃፍ የማይችሉ ከሆነ ማንሀብና መፃፍ የሚችል ገለልተኛ ምስክር፤ (ቢቻል ምስክሩ በተሳታፊው/ዋ ቢጠቆሙ ይመረጣል። በተጨማሪም ከጥናቱ ባለሙዎች ጋር ግንኙነት ሊኖራቸው አይገባም)
- የምስክሩ ስም፤ ----------
- የምስክሩ ፊርማ፤ ---------- ቀን፤ ----------

ለተመራማሪ/ለሐኪም

- ተሳታፊው/ዋ በዚህ ጥናት ለመሳተፍ ከመወሰኑ/ኗ በፊት በቂ መረጃና ማብራሪያ በሚገባው/ት ቋንቋና መንገድ እንዲያገኝ/እንድታገኝ አድርጊያለሁ። ስለሆነም ተሳታፊው/ዋ

ጉዳዩን በሚገባ እንደተረዳ/ችው እርግጠኛ ነኝ፤ 5-8 ሚሊ ሊትርየደም፡ጥቂትየሽንት፤የአክታ ወይም የቁስል ናሙናም እንድንወስድ ፈቅዷል/ፈቅዳለች።

- የተመራማሪው ስም፤ ----------
- የተመራማሪው ፊርማ፤ ---------- ቀን፤ ----------

በማወቅ የሚሰጥ የፈቃደኝነት ቅፅ ለወላጅ/አሳዳጊ(ከ12 አመት በታች)

ከተመረጡ የምርመራ ናሙና ውስጥ በሽታ በሚያመጡ የባክቴሪያ አይነቶችን መለየ፤የፀረ ባክቴሪያመዳኒትየመቋቋምባህሪያቸውን ማወቅ፤ ብሩክሆልድሪያ ሲዶማሊ የተባለውን ባክቴሪያ ጨምሮየዳሰሳ ጥናት ለሚሳተፉ የተዘጋጀ ቅፅ

የድርጅቱ ስም፡-አዲስ አበባዩኒቨርሲቲ የጤና ሳይንስኮሌጅ የህክምና ላብራቶሪሳይንስ ዲፓርትመንት እናአርማወርሀንሰን የምርምር ተቋም

የስፖንሰሩ ስም ፡- አርማወር ሀንሰን የምርምር ተቋም

አርስት

በሚካሄደው ከተመረጡ የምርመራ ናሙና ውስጥ በሽታ በሚያመጡ የባክቴሪያ አይነቶችን መለየት፤ የፀረ ባክቴሪያመዳኒትየመቋቋምባህሪያቸውን ማወቅ፤ ብሩክሆልድሪያ ሲዶማሊ የተባለውን ባክቴሪያ በተሻሻለ የባክቴሪያ ማሳደግ እና ከአፈር የዳሰሳ ጥናት

አጠቃላይ መረጃ

በጥናቱለመሳተፍ ከመወሰንዎበፊትይህንቅፅበትክክልአንብቡወይምሲነበብልዎበትክክልያዳምጡ፤ እንዲሁምግልጽያልሆነልዎትንነገርበሙሉነፃነትይጠይቁ።

ስለጥናቱመረጃ

የሳንባ ምች፣ የደም መቆሸሸሽ፣ የቆዳ ኢንፌክሽን እናየሽንት ቧንቧኢንፌክሽን ህመሞች በህመምተኛው ላይ የተለያዩ ችግሮችን ሊያስከትሉ ይችላሉ። ለምሳሌ የህክምናወጭንይጨምራል፣ ለህመምተኛውምቾትይነሳል፣ሆስፒታልውስጥ

ተኝቶየመታከምጊዜንያራዝማልብሎምለሞትሊዳርግይችላል።ስለዚህምየሳንባ ምች፣ የደም መቆሸሸሽ፣ የቆዳ መመርቀዝ እናየሽንት ቧንቧመመርቀዝ ህመምሊያመጡየሚችሉባክቴሪያዎችብሩክሆልድሪያ ሲዶማሊን (ሜሎዶሲስ የሚባል በሽታ አምጭ ባክቴሪያን) ጨምሮ የበሽታውን ምንጭ ማወቅህመሙንለመቆጣጠርናለመከላከልይጠቅማል።

የጥናቱ ዓላማ

የዚህ ጥናት ዓላማ ሜሎዶሲስን ጨምሮ የሳንባ ምች፣ የደም መቆሸሸሽ፣ የቆዳ መመርቀዝ እናየሽንት ቧንቧመመርቀዝ የሚያመጡ በሽታዎችን በአዲስ አበባ እና በባህር ዳር እንዲሁምአካባቢያቸው ምን ያህል እንደሚታዩ ማወቅ ነው። ከጥናቱ የሚገኘው መረጃ ለወደፊት ሜሎዶሲስን ጨምሮ የሳንባ ምች፣ የደም መቆሸሸሽ፣ የቆዳ መመርቀዝ እናየሽንት ቧንቧ መመርቀዝ የሚያስከትሉ በሽታዎችን ለማከምና ለመቆጣጠር ይረዳል።

ሜሎዶሲስብሩክሆልድሪያ ሲዶማሊን በሚባል ባክቴሪያ አማካኝነት የሚመጣ በሽታ ሲሆን በዋናነት የሚተላለፈው በንክኪ፣ በአየርእና በተበከለ ምግብ አማካኝነት ነው። በሽታው ከቀላል እስከ ከባድ ህመም የሚያደርስ ሲሆን ብሎም ለሞት ሊዳርግ ይችላል።

በጥናቱ ልጅዎ እንዲካተት ከወሰኑ መደረግ ያለበት

በጥናቱ ልጅዎ እንዲካተት ከወሰኑ አንዳንድ ጥያቄዎች እንጠይቅወታለን። ከጥያቄዎቹ መካከል፤ የልጅዎን ስም፤ የልጅዎንዕድሜ፤ የልጅዎን ጾታ፤ የልጅዎን ብሄርና ስለልጅዎህመም ሁኔታ ይገኙበታል። በተጨማሪም የልጅዎን በሽታ ለማስታገስ ምናልባት የወሰዳቸው መድሃኒቶች ስለመኖራቸው እንጠይቅወታለን።እንዲሁምለምርመራ ከተሰጠው ናሙና በሽታ ያመጣው ባክቴሪያ ለተጨማሪ የባክቴሪዮሎጂ እና የዘረመል ምርመራ እንወስዳለን፤ ውጤቱም እንደደረሰ የሚገለፅ ይሆናል። ምርመራውም ስም በሌለው በተለዬ ኮድ ይካሄዳል።

ለጥናቱ ተሳታፊዎች ያለው ጥቅም

በጥናቱለሚሳተፉፍቃደኛተሳታፊዎችምንምአይነትየገንዘብክፍያየለም።

ነገርግንልጅዎበምርመራውውጤትመሰረትየመታከምእድልይኖሮታል።

በተጨማሪምየጥናቱውጤትየሳንባምች፣የደምመቆሸሸሽ፣የቆዳመመርቀዝ

እናየሽንትቧንቧመመርቀዝለመቆጣጠርናለመከላከልስለሚጠቅምበተዘዋዋሪመንገድልጅዎለሌላህመም ተኛእንዲሁምህብረተሰቡንየሞጥቀምእድልያገኛል፡

በጥናቱተሳታፊዎችላይያለውጉዳት

ደምበሚወሰድበትጊዜበመርፌምክንያት በልጅዎየሚከሰትመጠነኛህመምወይምመበለዝሊኖርይችላል፡ ፡ይህምከጥቂትጊዜበኋላየሚጠፋሲሆንሌላይህነውየሚባልጉዳትአይኖርም፡፡ ናሙና ከቁስል ላይ ሲወሰድበልጅዎላይ ከመጠነኛ ስሜት በስተቀር በጤናው/ዋ ላይ ምንም ጉዳት አይደርስም፡፡ሽንትና አክታ ሲወሰድበጤናው/ዋ ላይ ምንም ጉዳት አይደርስም፡፡ የልጅዎምስቲራዊነትያላቸውመረጃዎችስለሚኖሩስጋትሊያድርብህ/ሽይችላል፡፡ ሆኖምየእነዚህመረጃዎችምስጢራዊነትበሚገባየተጠበቀነው፡፡2-4 ሚሊ ሊትርየደም፡ጥቂትየሽንት፤ የአክታ ወይም የቁስል መውሰድእንዲሁምከላይየተገለፁትንምርመራዎችናመጠይቆችማድረግምናልባትመጠነኛየምቾትመጓደ ልሊያስከትልብህ/ሽይችላል፡፡

የመረጃሚስጥራዊአጠባበቅ

የልጅዎሊለይየሚያስችልመረጃበሙሉበሚስጥርይያዛል፡፡በዚህጥናት ከልጅዎየሚሰበሰበውመረጃበሙሉየኮድቁጥርየሚሰጠውሲሆንምንምዓይነትስምአይመዘገብም:: የዚህኮድመፍቻበፋይልተቆልፎየሚቀመጥሲሆንየተፈቀደለትሰውብቻፋይሉንማየትይችላል፡፡ ከዚህጥናትበሚወጡዘገባዎችወይንምየህትመትውጤቶችላይስምንወይምሌላልጅዎንሊያስለይየሚያስ ችልመረጃአይኖርም፡፡

ከምርመራየሚገኝውምውጤትወይምሌላመረጃለሚመለከታቸውለምሳሌልጅዎንየሚንከባከቡየህክም ናባለሙዎችእናጥናቱንለሚያካሄዱትባለሙያዎችእንዲሁምጥናቱስነምግባርንጠብቆመከናወኑንለሚከ ተሉየመንግስትአካለትብቻይገለፃል፡፡

ጥናቱን እንቢ ማለት ወይንም ቋረጥ መብት

ልጅዎ ወይም አርስዎበዚህ ጥናት ያለመሳተፍ ወይንም ከጥናቱ አቋርጦ የመውጣት ሙሉ መብት አለወት፡፡

ልጅዎ ወይም አርስዎ ጥያቄዎችን አለመመለስ ምርመራዎቹንና ደምመውሰዱንም እንቢ ማለት ወይም የእረፍት ጊዜ መውሰድ ትችላለችሁ።በፈለጋችሁበት ጊዜም ከነጭራሹ ራሳችሁን ከጥናቱ ማግለል ትችላለችሁ::

ማንኛውም ዓይነት ጥያቄ ቢኖርዎት

ማንኛውም ዓይነት ጥያቄ ቢኖርዎት አሁን ወይም በማኝኛውም ጊዜ መጠየቅ ይችላሉ። በሌላ ጊዜ ጥያቄ ቢኖርዎት በዚህ አድራሻ ሊያገኙን ይችላሉ።

እማዋይሽ አንዳርጌ፤በስልክ ቁጥር 0911345215

እና አለርት/አህሪ ኢቲክስ ኮሚቴ 0118-962183

<u>ለህጻን/ለታዳጊወጣትየጥናትተሳታፊበወላጅ/አሳዳጊየሚሰጥየፈቃደኝነትቃል</u>

በአዲስአበባእናበባህርዳርእንዲሁም በሚካሄደው ከተመረጡ የምርመራ ናሙና ውስጥ በሽታ በሚያመጡ የባክቴሪያ አይነቶችን መለየ፤የፀረ ባክቴሪያመዳኒትየመቋቋምባህሪያቸውን ማወቅ፤ ብሩክሆልድሪያ ሲዶማሊ የተባለውን ባክቴሪያ በተሻሻለ የባክቴሪያ ማሳደግ እና ከአፈር የዳሰሳ ጥናት ለሚሳተፉ ከተዘጋጀ ቅፅ የፈቃደኝነት ቅፅን አንብቤያለሁ ወይምን ተነቦልኛል።

- ዓላማውና የአሰራሩ ሁኔታ&የጥናቱ ጥቅሞችና ሊኖሩ የሚችሉ ስጋቶች በሙሉ በተሟላ ሁኔታ ተገልፆልኛል።
- ጥያቄዎችን እንድጠይቅ ተፈቅዶልኛል እንዲሁም ለጥያቄዎቹ በጥናቱ ባለሙያዎች አጥጋቢ መልስ አግኝቻለሁ። ጥያቄ ቢኖረኝ ወይም የከነከኑኝን ነገሮች ካሉ ማንን ማናገር እንደምችል ተነግሮኛል::
- የተፈረመና ቀን የተፃፈበት ይህ የፈቃደኝነት ቅፅ እንደሚሰጠኝና በጥናቱ የሚገኙ መረጃዎች ሚስጥራዊነት እንደሚጠበቅና ከጥናቱ ዓላማ ውጪ እንደማይውል እንዲሁም ጥናቱን ከሚያካሂዱት ተቋማት ውጪ እንደማይወጣ መተማመኛ ተሰጥቶኛል::
- ስለሆነም በዚህ ጥናት ውስጥ ልጄ እንዲሳተፍ ፈቅጃለሁ። የጥናቱ ባለሙዎች የሚሰጡኝን መመሪያ እከተላለሁ እንዲሁም ሙሉ በሙሉ ከእነርሱ ጋር እተባበራለሁ።
- በማናቸውም ጊዜ ከጥናቱ ልጄን ማግለል እንደምችልና ይህም የሚደረግለትን ህክምና እንደማይለውጥ አውቄያለሁ። በዚህ ጥናት ልጄ ባለመሳፉ የሚኖር ቅጣት ወይም የሚጓደል ጥቅም አለመኖሩን አውቃለሁ።
- እኔ “ከተመረጡ የምርመራ ናሙና ውስጥ በሽታ በሚያመጡ የባክቴሪያ አይነቶችን መለየ፤ የፀረ ባክቴሪያ መዳኒት የመቋቋም ባህሪያቸውን ማወቅ፤ ብሩክሆልድሪያ ሲዶማሊ የተባለውን ባክቴሪያ ጨምሮ ዳሰሳ”ጥናት ልጄእንዲሳተፍ ተስማምቸ ከታች ፈርሜለሁ

ለተሳታፊ ወላጅ/አሳዳጊ

- የተሳታፊ ህጻን/ታዳጊ ወጣት ስም ፡- _____
- የወላጅ ወይም ያሳዳጊ ሰም:- _____
- የወላጅ/አሳዳጊ ፊርማ/የጣት አሻራ፤ ----------
- ቀን፡ ----------

ለምስክር

- የተሳታፊው/ዋ ወላጅ/አሳዳጊ ማንብብና መፃፍ የማይችሉ ከሆነ ማንሀብና መፃፍ የሚችል ገለልተኛ ምስክር፤(ቢቻል ምስክሩ በተሳታፊው/ዋ ወላጅ/አሳዳጊ ቢጠቀሙ ይመረጣል። በተጨማሪም ከጥናቱ ባለሙዎች ጋር ግንኙነት ሊኖራቸው አይገባም)
- የምስክሩ ስም፤ ----------
- የምስክሩ ፊርማ፤ ----------ቀን፤ ----------

ለተመራማሪ/ለሐኪም

- የተሳታፊው ወላጅ/አሳዳጊ በዚህ ጥናት ልጃቸው እንዲሳተፍ ከመወሰናቸው በፊት በቂ መረጃና ማብራሪያ በሚገባቸው ቋንቋና መንገድ እንዲያገኙ አድርጊያለሁ። ስለሆነም የተሳታፊው ወላጅ/አሳዳጊ ጉዳዩን በሚገባ እንደተረዱት እርግጠኛ ነኝ። 2-4 ሚሊ ሊትርየደም፡ጥቂትየሽንት፤ የአክታ ወይም የቁስል ናሙናም እንድንወስድ ፈቅደዋል።
- የተመራማሪው ስም፤ ----------

- የተመራማሪው ፊርማ፤ ---------- ቀን፤ ----------

Annex 3.Recruitment Form

I. Study site information

1.Reporting site	ALERT Centre ◯	Tikur Anbessa H.◯	Felege Hiwot H.◯
2.Date of visit	__ __/__ __/__ __ __ __DD/MM/YYYY		
3.Recruitment site	Inpatient department◯	Outpatient department◯	Pediatric ward◯

II. Participant information

1.Informed consent obtained	Yes ◯	No◯
2.Participant ID		
3.full Name		
4.Sex	Male ◯	Female◯
5.Age		
6.Address		
7.Occupation		

III. Interviewer/Treating physician

All form sections completed

Name:_____

Signature:_____

Annex 4. Case Report Form

I.Participant information

1. Participant ID _____

II. History of current illness

1. Onset of illness _____
2. Symptoms (multiple answers possible)

2.1.**Symptoms of pneumonia:-**Fever, sweating and chills ◯Cough ◯Chest pain during breathe or cough◯ Shortness of breat ◯Fatigue ◯ Nausea, vomiting or diarr hea ◯Other symptoms_____

2.2.**Symptoms of Sepsis:-**Fever◯chills and shivering ◯ fast heartbeat◯ fast breathing ◯ ,Other symptoms_____

2.3.**Symptoms of Skin infections:-**Boils ◯Impetigo ◯ Cellulitis ◯

Other symptoms_____

2.4.**Symptoms of urinary tract infection:-** Pain or burning feeling during urination ◯feeling of urgency ◯altered appearance of the urine◯ Passing only a tiny amount of urine ◯,Other symptoms_____

3. History of medication prior to current medical evaluation

3.1.No ◯
3.2.Yes ◯

III.Interviewer/Treating physician

All form sections completed ◯

Name: _____Signature: _____Date:_____

Annex5. Laboratory Form

I.Participant and sample collection information (to be completed at Hospital/Clinic)

1. Reporting study site _____
2. Participant ID _____
3. Gender ,male ◯ Female ◯
4. Age_____
5. Type of specimen collected_____
6. Date and time sample taken __ __/__ __/__ __ __ __(DD/MM/YYYY)
   __ __:__ __ (hh:mm) Am ◯Pm ◯
7. Specimen collected by: Name: _____ Designation: _____

II.Culture (to be completed at Laboratory)

1. Date and time sample received in the lab

__ __/__ __/__ __ __ __ (DD/MM/YYYY)

__ __:__ __ (hh:mm) Am ◯Pm ◯

Inoculate __ __/__ __/__ __ __ __ (DD/MM/YYYY)

__ __:__ __ (hh:mm) Am ◯Pm ◯

Blood agar ◯MacConkey agar ◯ Chocolate agar ◯ Ashdown Agar ◯other spesify_____, _____

2. Growth result within _____hour Positive ◯Negative ◯
3. If growth positive ,gram stain:Gram +ve ◯Gram -ve ◯
4. Growth Characteristics

Blood agar

_____

_____

_____

MacConkeyagar

_____

_____

_____

Chocolateagar

_____
_____
_____

AshdownAgar

_____
_____
_____

Other media specify

_____
_____
_____
_____

5. Identification tests depend on colony Characteristics and gram stain

_____
_____
_____
_____
_____
_____
_____
_____
_____

6. Identification of isolate/ isolates: _____,_____
7. Drug susceptibility pattern
    a. Primary isolate :sensitive to _____

        Intermediate to _____

        Resistance to_____

        Sceond isolate identified: sensitive to _____

        Intermediate to _____

        Resistance to_____

Name _____

Signature_____

Date_____

Annex 6. Laboratory principle, procedure and culturing of clinical sample

I. Laboratory procedure for collection and culturing of clinical sample

Pus:

1. Wherever possible a specimen of pus/fluid collected aseptically into a sterile universal container is preferred over a swab.
2. It is important to accurately record the anatomical site of a swab specimen, since cultures from swabs of a deep pus collection will require a different interpretation to those from a superficial site.
3. Label the sample as soon as possible with the patient code number
4. Label petri dishes(BAP, MacConkey and Ashdown Agar)
5. Inoculate in to BAP and MacConkey agar aseptically and incubate the plate aerobically at 35-37^{0}C for 18-24 hours, paralleley incubate on Ashdown Agar at 37-42°C for up to 96hours.
6. Examine and report the culture; look for colony characteristics gram reaction and perform biochemical.
7. Determine drug susceptibility pattern of the isolated organism.

Blood

1. use proper skin disinfection for obtaining blood for cultures to minimize contamination with skin bacteria, and obtaining sufficient blood for culture
2. Label the sample as soon as possible with the patient code number
3. If use automated blood culture system ,positive flagged remove it from the automat and transfer it to a biological safety cabinet
4. Label petri dishes (blood agar, chocolate agar, MacConkey agar)
5. Label a slide
6. Incubate over night at 36±1°C an enrichment mediumif positive subculture on blood agar and MacConkey Agar under aerobic conditions, chocolate agar in CO2. If CO2 incubator not available use candle jar or another device.

7. Document the result of the gram stain as the first step of identification
8. Examine and report the culture; look for colony characteristics gram reaction and perform biochemical
9. Determine drug susceptibility pattern of the isolated organism
10. If notuse automated blood culture systemdirectly incubate over night at 36±1°C an enrichment mediumif positive subculture on blood agar and MacConkey Agar under aerobic conditions, chocolate agar in CO2. If CO_2 incubator not available use candle jar or another device.

Urine

1. A clean-catch midstream urine samplewill be collected for testing. This method helps protect the urine sample from microbial floras around the genitalia.
2. Label the sample as soon as possible with the patient code number
3. Label petri dishes(BAP, MacConkey and Ashdown Agar)
4. Urine will be inoculated into MacConkey agar and bloodagar; the plates incubated over night at 35°C -37°Cand on Ashdown Agar at 37-42°C for up to 96 hours.
5. Examine and report the culture; look for colony characteristics gram reaction and perform biochemical.
6. Determine drug susceptibility pattern of the isolated organism

Sputum:

1. Sputum samples that are coughed up or expelled into a sterile cup provided by the laboratory. Deep coughing is generally required, and the person should be informed that it is phlegm/mucus from the lungs that is necessary, not saliva.
2. Label the sample as soon as possible with the patient code number
3. Label petri dishes(BAPand Ashdown Agar)
4. Sputum will be inoculated into blood agar, at 35°C -37°C over night and on Ashdown Agar at 37-42°C for up to 96 hours.
5. Examine and report the culture; look for colony characteristics gram reaction and perform biochemical.
6. Determine drug susceptibility pattern of the isolated organism

Laboratory principle and procedure for Gram staining technique

Principle

Bacteria can be classified upon their cell walls. Bacteria with a high amount of peptidoglycan will retain crystal violet upon decolorization with 10% acetone-alcohol. They appear purple. Gram negative bacteria have a lower amount of peptidoglycan and cannot retain crystal violet. Counterstain with safranin or carbol fuchsin will color these bacteria pink to red[49].

Procedure

1. Labeling the slides clearly with the date and patient's name and number.
2. Making of smears by spread evenly covering an area about 15-20mm diameter on a slide.
3. Drying of smears after making smears, the slide should be left in a safe place to air-dry, protected from flies and dust.
4. Fix the dried smear by using heat or chemicals (methanol).
5. Cover the fixed smear with crystal violet stain for 30-60 seconds.
6. Rapidly wash off the stain with clean water. If the tap water is not clean, use filtered water or clean boiled rainwater.
7. Tip off all the water, and cover the smear with lugol's iodine for 30-60 seconds.
8. Wash off the iodine with clean water.
9. Decolorize rapidly (few seconds) with acetone alcohol. Wash immediately with cleanWater.
10. Cover the smear with neutral red or safranine stain for 2 minutes.
11. Wash off the stain with clean water.
12. Wipe the back of the slide clean, and place in a draining rack for the smear to air-dry.
13. Examine the smear microscopically, first with the 40 X objective to check the staining and distribution of the analytes and, then turn to the oil-immersion objective to lookfor bacteria and cells.

Result Intrpritation

- Gram positive bacteria ----------dark purple
- Gram-negative bacteria ----------pale to dark red

Control

- Gram positive control---------- *E. coli*: gram negative rods
- Gram-negative conttol----------*S. aureus*: gram positive cocci

III. Identification of *Burkholderia pseudomallei* from clinical specimen

Principle

The principle is based on the growth of *B. pseudomallei* from clinical specimens on selective solid media (Ashdowns agar). Identification of colonies is based on a simple screening system involving gram staining, the oxidase reaction, testing colistin and amoxicillin/clavulanic acid resistance and typical colony morphology**[8].**

Procedure

1. Collect clinical Sample
2. Place > 50 µl of the material on Ashdown Agar and Incubation at 37-42°C for up to 96hours and check for growth every day
3. Growth on Ashdown? See Purple & wrinkled dry colonies with metallic sheen?Subcultivation on LB or other unselective agar
4. Identification of isolates

4.1.Emulsify one medium sized colony in 3 ml of normal saline

4.2.Use a cotton swab to cover an LB-Agar plate with emulsified *B. pseudomallei*

4.3.Let the plate dry for 5 to 10 minutes at room temperature

4.4.Use sterile forceps to place a Amoxicillin/Clavulanic acid and Collistin containing disc on agar plate

4.5.Incubate test plates for 24h at 37°C

4.6.Measure diameter of inhibition zone around antibiotic discs

4.7. *B. pseudomallei* should exhibit the following inhibition zones

4.8*B. pseudomallei* should exhibit the following inhibition zones:

- Amoxicillin/Clavulanic acid >20 mm (susceptible)
- Collistin no inhibition zone (or below 9mm)

IV. Laboratory procedure for Biochemical testing

Biochemical tests for gram positive bacteria: Gram-positive cocci will be identified based on their gram reaction, catalase and coagulase tests results.

Catalase test

Purpose: Catalase test to differentiate staphylococci which produce the enzyme catalase from streptococci which are non catalase producing.

Principle

Catalase is an enzyme that will increase the speed of the following reaction:
2H2O2 → 2H2O + O2↑. Catalase is present in most cytochrome containing aerobic and facultative anaerobic bacteria, with the exception of streptococci. Presence of the enzyme can be made visible by addition of H2O2 to the suspected colony. In the presence of catalase the forming molecular oxygen is visible as little bubbles**[49].**

Procedure

1. Add few drops of 3% (v/v) H2O2 to a microscopic slide.
2. With a sterile wooden applicator stick immerse several colonies in H_2O_2. Do not immerse agar with the colonies.
3. Read and record result on appropriate data sheet.

Interpretation of Results:

- Development of bubbles is a positive result

Cntrol:

- Positive control: *Staphylococcus* species
- Negative control: *Streptococcus* species

Coagulase test

Purpose:To differentiate *Staphylococcus aureus* (coagulase positive) from the coagulase negative species e.g. *S. epidermidis and S. saprophyticus*

Principle

There are 2 forms of coagulase: one bound to the cell wall (bound coagulase – clumping factor) and one liberated by the cell (free coagulase). While the slide coagulase test determines the presence of bound coagulase, the tube test detects both bound and free coagulase. The exact mechanism by which the enzyme coagulase coagulates plasma is not known. It is generally accepted that a plasma factor is involved and that it resembles prothrombin**[**53**].**

Procedure

Slide coagulase test:

1. On a slide make a heavy suspension of the S*taphylococcus* to be tested in saline
2. Observe for auto-agglutination. If auto-agglutination occurs, slide test cannot be performed, set up tube coagulase test.
3. Add a loopful of fresh coagulase plasma.
4. Mix with saline suspension while observing for appearance of immediate (maximal within 20 seconds) clumping. This is a positive test.

Tube coagulase test

1. Make a heavy suspension of the *staphylococcus* to be tested in 2 ml of broth (e.g. Brain Heart Infusion, Trypticase Soy Broth).
2. Add 0.4 ml of coagulase plasma.
3. Incubate at 37^{o} C. Observe appearance of coagulum after 2 and 4 hours by gently tilting tube; avoid agitation.

4. Incubate overnight if negative after 4 hours.

Interpretation of Results:

Slide Coagulase:

- Clumping/agglutination of bacteria - Positive
- No clumping/agglutination of bacteria - Negative

Tube Coagulase Test:

- Formation of clot - Positive
- No clot formed - Negative

Controls

- Positive control: *Staphylococcus aureus*
- Negative control: *Escherichia coli*

Biochemical test for gram negative bacteria: - Identification of gram negative bacteria will be based on their test result with a series of biochemical tests.

Procedure

1. Prepare a suspension of the test organism with nutrient broth. 3-4 colony of test organism in5 ml nutrient broth.
2. A loop full of the bacterial suspension is inoculated in to KIA, indole, citrate agar, triple sugariron agar, lysine decarboxylase agar, manitol, urea agar, oxidase and motility medium.
3. Incubate at 35-37 Oc for 18-24 hours
4. Look for color change (turbidity for motility) of the medium
5. Identify the test organism by considering the result of the ten biochemical tests or EPI 20E.

II. Laboratory procedure for Antimicrobial sensitivity testing

Principle Mueller Hinton Agar

Beef Extract and Acid Hydrolysate of Casein provide nitrogen, vitamins, carbon, and amino acids in Mueller Hinton Agar. Starch is added to absorb any toxic metabolites produced. Agar is the solidifying agent. A suitable medium is essential for testing the susceptibility of microorganisms to sulfonamides and trimethoprim. Antagonism to sulfonamide activity is demonstrated by para-aminobenzoic acid (PABA) and its analogs. Reduced activity of trimethoprim, resulting in smaller growth inhibition zones and inner zonal growth, is demonstrated on medium possessing high levels of thymide. The PABA and thymine/thymidine content of Mueller Hinton Agar are reduced to a minimum, reducing the inactivation of sulfonamides and trimethoprim**[50].**

Procedure

1. Emulsify several colonies of similar appearance of test organism in small volume ofnutrient broth.
2. Match the turbidity of the suspension against the turbidity standard which has a similar appearance to an overnight broth culture.
3. With a sterile swab take sample from the suspension (squeeze the swab against the side of the test tube to remove the excess fluid).
4. spread the inoculum evenly over the Muller-Hinton agar plate with the swab
5. Using a similar inoculation technique, inoculate an overnight broth culture of the Control organism evenly across the upper and lower third of the plate.
6. using a sterile forceps or needle ,place the antimicrobial disc on the inoculated plate
7. incubate the plate aerobically at 35-37oC For 18-24 hours
8. Read the tests after checking that the bacterial growth of the test and control organism isneither too heavy nor too light
9. Measure the radius of the inhibition zone. interpret the reaction of the test organism to each antibiotics used as sensitive, intermediate, or resistance as per the standard

Sensitivity (S): Zone of radius is wider than, equal to, or not more than 3mm smallerthan the control.

Intermediate (I): Zone radius is more than 3mm smaller than the control but not less than 3mm.

Resistant (R): No zone of inhibition or zone radius measure 2mm or less.

Declaration

I, the undersigned, declare that this M.Sc. research thesis is my original work, ithas not been presented for a degree in this or any other university and that all sources of materials used for the research proposal have been duly acknowledged.

M.Sc. candidate: **Emawayish Andargie(BSc)**

Signature: _____

Date of submission: _____

Place Addis Ababa, Ethiopia.

This research thesis has been submitted with our approval as advisors.

Name of advisor: Kassu Desta (MSc, PhD candidate)

Signature _____

Place: Department of Medical Laboratory Sciences, Addis Ababa University

Date of submission_____/_____/_____