MOLECULAR DETECTION OF GROUP B STREPTOCOCCUS IN CULTURE NEGATIVE INFANTS SUSPECTED OF MENINGITIS AT TIKUR ANBESA SPECIALIZED HOSPITAL

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ABSTRACT

Background: Meningitis is an infection of the meninges, characterized by an onset of fever, headache, neck stiffness, and photophobia over a period of hours to days. In Ethiopia, meningitis due to an infectious agent is among the top ten causes of death among infants. The rate of maternal and neonatal Group B streptococcus (GBS) colonization is high that contribute to acquisition of meningitis. However, there is study gap to rule out GBS meningitis in Ethiopia where its magnitude is unknown. Therefore, this study was aimed to determine the magnitude of GBS in infants with suspected meningitis.

Methods: Hospital based cross sectional study design was implemented for identification of GBS in infants with suspected meningitis at Tikur Anbessa specialized hospital by using PCR targeting *cfb* gene encoding the Christie-Atkinson-Munch-Peterson factor (CAMP) from June 2018 to October 2018. All the CSF samples were cultured on BHI, chocolate, blood agar plates and MacConkey. Analysis was done using SPSS version 25.

Results: The CSF culture was found all negative. However, the magnitude of GBS was 63.9 % (46/72) through cfb targeted PCR. Out of the 46 GBS positive infants, 10.9% (n=5) of them died. The late onset of GBS (LOGBS) disease was noted to have poor outcome with 3 LOGBS out of 5 GBS positive deaths occurred. Outcome of infants were found related with onset disease.

Conclusion and recommendations: The *cfb* gene targeted PCR contributes a lot for identification of GBS in culture negative cerebrospinal fluid (CSF) samples and hence this more sensitive technique needs to be conducted at least at the referral hospitals.

Key words: Meningitis, GBS, infant, bacterial meningitis, Ethiopia

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ABBREVIATIONS

AHRI	Armauer Hansen Research Institute
ALERT	All African Leprosy Rehabilitation and Training center
AOR	Adjusted Odds Ratio
ATCC	American Type Culture Collection
BBB	Blood Brain Barrier
BM	Bacterial Meningitis
CAMP	Christie-Atkinson-Munch-Peterson
CDC	Centeres for Disease Control and Prevention
CI	Confidence interval
CNS	Central Nervous System
COR	Crude odds ratio
CRP	C- reactive protein
CSF	Cerebrospinal fluid
DNA	Deoxyribonucleic Acid
EDHS	Ethiopian Demographic and Health Survey
EOD	Early Onset Disease
EPHI	Ethiopian Public Health Institute
GBS	Group B streptococcus

IAP	Intra-partum antibiotic prophylaxis
JUMC	Jimma University Medical Center
LOD	Late Onset Disease
LP	Lumber puncture
OR	Odds Ratio
PCR	Polymerase chain reaction
SSA	Sub-Saharan Africa
TASH	Tikur Anbessa Specialized Hospital
ULOD	Ultra Late Onset Disease
μM	Micro-molar
μl	Micro-liter
WBC	White Blood Cell
WCC	White Cells Count
WHO	World Health Organization
X^2	Chi –square

1. INTRODUCTION

1.1. Background

Bacterial meningitis (BM), is an inflammation of meningeal membranes lining the brain and spinal cord by bacterial infections (1) . *Neisseria meningitidis*, *Streptococcus pneumoniae* and *Haemophilus influenzae* were the most common causes of BM in infants and adults. Introduction of vaccines against these bacterial etiologic agents of meningitis have markedly decreased their incidence (2, 3). On the other hand, *Streptococcus agalactiae* (known as group B Streptococcus (GBS)) and *Escherichia coli (E. coli) K1* strain remain predominant pathogens causing BM in febrile infants as both do not have public administrable vaccine (4), and GBS ruins first.

Group B streptococcus is an extracellular Gram-positive, catalase negative encapsulated coccus with beta (β) - hemolytic activity on blood agar and resistant for bacitracin. Based on their capsular polysaccharide ten serotypes are recognized to exist (Ia, Ib, II-IX). Of these diverse variants, five (Ia, Ib, II, III, and V) are basically related to disease globally and serotype III that belongs to multi-locus sequence 17 is the most common cause of BM in infants (5, 6). Maternal colonization is the primary means of newborns GBS acquisition either via ascending infection or during birth through infected birth canal as neonates aspirate contaminated amniotic or vaginal fluids (7). About 20-30 % of healthy women inhabited GBS on their vaginal or rectal mucosa (5). Mostly early onset diseases are due to the ascending spread of the organism into the amniotic fluid or during passage through birth canal (8).

The invasive GBS infections can be categorized as early onset disease (EOD) and late onset disease (LOD) and rarely as ultra-late onset disease (ULOD) based on its occurrence to the age of the infant during onset of disease. Early onset of disease is defined as the onset of disease within the first six days of life, while LOD is the occurrence of disease after the first week of life (up to 89 days after birth, usually within first month after birth). However, the disease onset may be prolonged up to 6 months or above (including ULOD, which occurs after 3 months of age) (9, 10).

Group B Streptococcus is known to encode different virulence factors that are essential for disease development. Of the diverse virulence factors; capsular polysaccharide and pore forming

toxins are the principal factors. Pore-forming toxins are the vital machineries of pathogenesis as it facilitate entry of the pathogen into host cells, support intracellular survival and systemic dissemination. The two pore forming toxins produced by *S. agalactiae* are β-haemolysin/cytolysin (β-H/C) and Christie Atkins Munch Peterson (CAMP) factor (11, 12). The genome analysis of five major disease causing GBS strains (Ia, Ib, II, III, and V) has shown the presence of core genome shared by all GBS strains (12).

Of the virulence determinants of GBS, *cfb* gene encoded CAMP factor has been used in diagnostic microbiology to identify the strains, as it produces a distinct zone of hemolysis on blood agar plates when grown near *Staphylococcus aureus* colonies (known as CAMP reaction) (13, 14). Therefore, targeting CAMP factor encoding gene is a novel to use as a diagnostic tool since the *cfb* gene is the core for all GBS strains including the CAMP factor negative strains.

1.2. Statement of the problem

Bacterial meningitis has remained to be a global challenge. The types and distribution of bacterial etiologic agents of meningitis in infants vary in relation to birth gestational age, age of infants, commonly highest during the neonatal period, and geographic location (1). GBS is not listed in the major etiological agents of infant sepsis and meningitis in most of Africa and the developing world (15). Consequently, health professionals may not be aware of the incidence of GBS versus other prevalent named bacterial etiologies in Africa. Detection of the specific causative agent of meningitis is always critical for patient management so as to reduce morbidity and mortality. This also adds value for better understanding in the epidemiology of the disease in the local setting in order to provide inputs for policy makers for disease prevention and control.

Rapid diagnosis is much critical for right selection and duration of antibiotics against bacterial meningitis. Cerebrospinal fluid (CSF) culture, the gold standard diagnostic tool of BM, could be compromised by antibiotic uptake prior to diagnosis. Usually, antibiotics are given to the infants before CSF collection and lead into false negative culture results. Reports have shown that more than 75% of the times lumber puncture (LP) is performed after initiation of antibiotics even in the developed countries to minimize complications and fatalities (1). Moreover, the relatively high frequency of traumatic tap in infants makes the data more challenging to interpret (16). This could result in empiric diagnosis and non- selective treatment of bacterial meningitis in infants.

In Ethiopian set up; the main causes of BM, both in adults and infants were *Neisseria meningitidis*, *Streptococcus pneumoniae* and *Haemophilus influenzae* (17). There is no report of neonatal and infantile meningitis due to GBS. However, there would be high potential of vertical transmission of GBS to newborns (18) as the maternal GBS colonization rate is high (vary from 14.6% (19) to 19% (20). The diagnostic tool in use may need some considerations. Because in one of the study conducted at Jimma, bacterial pathogens responsible for meningitis were detected in one third of cases using PCR based system of which nearly all (97%) were negative by culture method (21), though the magnitude of meningitis due to GBS among infants is unknown.

Recently GBS emerges as a leading cause of meningitis in neonates and young infants, resulting with high mortality (22). The burden of meningitis at Tikur Anbessa Specialized Hospital (TASH) varies from 4.7% (23) to 35% (17) though the specific contribution of GBS is not shown. Since Ethiopia focused more on other bacterial causes of meningitis, GBS caused meningitis may end up with unpredictable consequences unless something is done. Therefore, estimating the contribution of GBS for neonatal and infantile meningitis is important.

1.3. Significance of the study

A study done in Hawassa showed that newborns from GBS colonized mother had a higher risk to acquire this pathogen with a higher vertical transmission rate (18). However, no reports about GBS meningitis in Ethiopia in which our study is intended to address. Therefore this assessment of GBS will explain the burden of GBS meningitis in the country both in terms of incidence and mortality among infants. Moreover, because epidemiologic pattern of bacteria could change with time and place, knowing the local condition would have paramount importance to devise prevention and control strategies at the local setting.

2. LITERATURE REVIEW

2.1. Overview of meningitis in infants

An infection and inflammation of meninges (meningitis) remains to be the main cause of morbidity and mortality in neonates and younger children throughout the world. However, the morbidity and mortality of meningitis varies with age, geographic location of patients and the etiologic organism (1, 24). Usually the onset of meningitis is considered early when happening during the first six days of life, late when occurring between 7 and 89 days, and very or ultra-late when happens beyond 3 months of life (25).

Generally the widespread use of conjugated vaccines against the major bacterial pathogens, has decreased the incidence and prevalence of associated bacterial meningitis. However, because of the shift in etiologic agents, especially in neonates and young infants, the burden remains high (3, 24). Reports has shown the emergence of the bacterial pathogens such as GBS (with less previous concern) as the most predominant cause of meningitis in young infants and neonates. Therefore, early diagnosis and effective antibiotic treatment are the keys for successful management of bacterial meningitis.

2.1.1. Neonatal meningitis

Neonatal meningitis is an infection and inflammation of meningeal sheath during the neonatal period, typically occurs between birth and the first 28 days of life. At this infantile period, meningitis is associated with a significant morbidity and mortality. Neonatal meningitis contributes to a considerably high mortality rate, ranging from 5-25% and about 25 to 50% of the survivors bear long term neurologic impairment and developmental delay (26, 27). Developing countries with poor settings and in accessibility of basic antenatal cares services worsen morbidity and mortality of neonates. Neonates with low birth weight, prematurity, immature immune system and poor maternal health conditions, are more prone to develop septicemia and consequently meningitis, which possibility results in death (28).

2.2. Causes of meningitis in infants

Meningitis can be caused by viruses, bacteria, fungi and parasites. Of these infectious organisms; bacteria and viruses are the predominant pathogens. *Neisseria meningitidis*, *Streptococcus pneumoniae*, *Haemophilus influenzae* type b, *Streptococcus agalactiae* (GBS), *E. coli* and *Listeria monocytogenes* have been the most common bacterial causes of meningitis. Human herpes viruses (HHVs), enteroviruses (EVs), and arboviruses are among the most common viral etiologies (29, 30). Parasites and funguses rarely causes meningitis. Fungal meningitis is a rare, life threatening disease and it can be caused by a variety of fungi. However, the most frequent fungal causes are *Cryptococcus neoformans* and *Candida albicans* and usually occurs in immuno-compromised patients

2.3. Risk factors

Predisposing risk factors for meningitis in neonates are preterm birth, low birth weight (<2500g), chorioamnionitis, endometritis, maternal GBS colonization, prolonged rupture of membranes, fetal hypoxia, galactosemia and previous history of hospitalization (31, 32). Similarly asplenia (anatomic or functional), Primary immunodeficiency, Human immunodeficiency virus (HIV) infection, Sickle cell anemia, CSF leak, recent upper respiratory tract infection, lack of breastfeeding, exposure to infected persons, penetrating head trauma and lack of immunizations are possibilities that make children more prone to acquire BM (32).

The risk of morbidity and mortality increased if the patients are newborns, live in low-income countries, infected with Gram-negative bacilli and *Streptococcus pneumoniae*. Additionally severe illness, infection with antimicrobial resistant organisms, and incomplete knowledge of the pathogenesis of meningitis are contributing factors to mortality and morbidity associated with BM (33). Predominance of GBS disease in neonates and infants, is associated with prematurity (<37 weeks gestation), low birth weight, mode of delivery (vaginal or caesarean section), maternal GBS colonization status, maternal intrapartum GBS antibiotic prophylaxis, breast milk feeding, maternal mastitis, previous GBS disease, sibling with GBS disease and urogenital abnormalities are the possible risk factors (25, 34, 35).

2.4. Diagnosis

Fever, headache, photophobia and neck stiffness are the classic symptoms of meningitis, used for suspicion. However, in early stages of meningitis, particularly in young children, the symptoms of meningitis can be variable and non- specific; making the diagnosis difficult with the classic symptoms. Studies have indicated that features such as bulging fontanel , neck stiffness, seizures or reduced feeds are suggestive about the presence of meningitis though not conclusive (36). Similarly a multicenter study from health facilities of Bangladesh, Bolivia, Ghana, India, Pakistan, and South Africa has developed a clinical diagnostic algorithm in infants under 2 months by assessing sensitivity, specificity, and odds ratio for each symptom and sign individually and combined into algorithms to evaluate their value for predicting severe illness like meningitis. Accordingly, sign and symptoms such as history of difficulty in feeding, temperature of 37·5°C or more and history of convulsions were shown as better predictor of severe illness with sensitivity (85%) and specificity (75%)(37).

In some cases patients with possible meningitis features may not have real meningitis. The Kenyan cohort was taken as evidence, provide 93% of cases with possible meningitis features has found not to have meningitis. In contrast, confirmed meningitis cases (26.7%) my also lack any clinical signs to differentiate septicemia from BM. The appearance of CSF could also be used for diagnosis of meningitis, provide in about 72% of bacterial meningitis cases the CSF samples looked normal and the probability of CSF cultures to be positive is about 30% (38).

C reactive protein (CRP) is an acute phase reactant protein, which usually increased during acute inflammatory or infectious process. Normally, serum CRP should not be detected or should be found less than 10 mg/L (39) . Usually, lumbar puncture (LP) is recommended when a C-reactive protein (CRP) level >10mg/L (40). The serum CRP sensitivity and specificity was 90.62% and 32.4% for pyogenic meningitis, and 64.7% and 24.52% for viral meningitis respectively. Similarly CSF CRP sensitivity and specificity was 96.87% and 74.73% for pyogenic meningitis, and 20.58% and 50.94% for viral meningitis respectively. In this study a CSF CRP of 4 mg/L and a serum CRP of 6 mg/L was considered positive and CSF CRP was more sensitive and specific for pyogenic meningitis than serum CRP (41).

Normally CSF had ≤5 white cells, glucose concentrations range 40-75 mg/dl and the average protein concentration ranges 40-100 mg/ dl (42, 43). However, in the cases of BM white cells are expected to raise above 100 cells/µl, protein elevated above 100mg/dl and glucose decreases below 40mg/dl (41, 44). The controversy is without the predicted decrement in glucose and elevation in protein concentrations and increment in white cell counts, meningitis may occur.

A study in china has shown that neonatal BM should not to be excluded even if the CSF WBC is within normal range (45). Similar study from china also used CSF pleocytosis (≥20 cells/mm3) as a diagnostic criteria of BM (46). Another study done in Australia, has indicated the value of CSF parameters in the early microbiological assessment of meningitis. According to this study, protein concentration below 60 mg/dl and WCC less than 90 cells/ µl were optimal cut-offs value for excluding BM (47). Based on our rough literature review and observations, including or excluding bacterial meningitis using western references is thought to be a challenge unless standardized to our setting. Because, in our study most of infants (79.6%) suspected with BM had white cells below 20 cells/ µl. Therefore, the gold standard CSF culture and if available more sensitive tools like PCR would be techniques of choice for diagnosis of GBS.

2.5. Bacterial meningitis

Unlike other etiologic organisms, bacteria caused meningitis is the major, serious and potentially life-threatening medical emergency with high case fatality and substantial after-effects (48). The beginning of conjugated vaccines against *Neisseria meningitidis*, *Streptococcus pneumoniae* and *Haemophilus influenzae* type b, has considerably reduced their associated burden (24, 48). Nowadays BM is switched by Group B streptococcus, followed by *Escherichia coli* and then *Listeria monocytogenes* (26). Particularly in neonates GBS and *E. coli* are the predominant pathogens. Hence, 50 to 60% of BM among neonates is caused by GBS, while around 20% of meningitis cases in neonates is caused by *Escherichia coli* (32).

2.6. Microbiology of Streptococcus agalactiae

2.6.1. General characteristics of *Streptococcus agalactiae*

Group B streptococcus (GBS), is a Gram-positive encapsulated bacterium that belongs to the group of pyogenic streptococci and it is the only streptococcus species harboring the Lancefield

group B cell-wall-specific polysaccharide antigen that is common to all GBS strains (49). Based on the type-specific capsular polysaccharides of GBS, their exists ten variants or serotypes (Ia, Ib, II-IX) (6). Most human GBS isolates grow readily on blood agar after overnight incubation with a narrow zone of beta-hemolysis. The simple and specific method to distinguish GBS from other beta-hemolytic streptococcal species is detection of the reddish pigment on Granada-type media. The absence of other streptococcal species that produce granadaene pigment make this pigment detection as a simple and fully specific method for single-step identification of GBS (49). Overall GBS is beta- hemolytic, catalase negative, CAMP positive, bacitracin resistant, hippurate positive and PYR negative test are the unique features for identifications of *S. agalactiae* (49).

2.6.2. Epidemiology of GBS meningitis

Group B *Streptococcus* (GBS) remains the most predominant pathogen responsible for meningitis in neonates and young infants in Europe, Australia, and North America (50, 51). In United Kingdom and Republic of Ireland during July 2010- July 2011, the annual incidence of bacterial meningitis in infants aged <90 days was 0.38 per 1000 live births, without any substantial difference by regions. In this study, the overall prevalence of GBS was 50% (150/302) with the case fatality rate of 5 % (7/135) (50). Similarly in Crete, Greece, the incidence of GBS disease in neonates and young infants was 0.17/1000 live births. In this study, the magnitude of GBS meningitis was 32%. Infants with LOD were more prone have meningitis than infants with EOD (44.4% vs. 25%). In Crete, Greece, EOD was not decreased when compared with other countries who have implemented prevention strategies (31).

In most developed nation, such as United States, the rate of GBS meningitis was 62.5% per 100,000 population in 2002-2007. During the 2003-2007 surveillance of bacterial meningitis in children, GBS caused 86.1 % of cases among infants under 2 months of age. The case fatality rate were 11.1% even after the introduction of universal GBS screening program to women. 86.5 % of cases were late onset GBS (51), not affected by the intrapartum antimicrobial prophylaxis (31, 51). Similarly in Canada, GBS accounted for 30.7% of the cases among infants (aged < 90 days) with bacterial meningitis in years 2013 and 2014. Accordingly, GBS was the leading cause 47.05 % (8/17)) of early-onset meningitis cases (52) .

In china, of the main cause of meningitis in children age from 28 days to 18 years old, GBS share was 10%. Whereas from the diagnosed meningitis cases in younger infants less than 3 months, GBS (46.5% (20/43)) was the leading organism identified followed by E. *coli* (23.3 % (10/43)) (44). Other multicenter retrospective cohort study in china (2005-2017), have identified GBS (*n* = 55, 29.1%) and *E. coli* (*n* = 55, 29.1%) as the leading causes of early-onset and late-onset neonatal meningitis, respectively (46). Whereas in Korea, GBS was responsible for 27.7% of the invasive cases among infants < 3 months of age. However, 69.0% of meningitis were attributed by GBS in this age group (53).

In Africa, sub-Saharan Africa in particular, had an average of 21.8% maternal GBS colonization across the region. Collective the most frequent disease-causing serotype was serotype, followed by 1a, 1b, II and V (54). In Malawi, GBS were accountable for 45% and 19.9% of early and late onset of meningitis cases. While, the overall magnitude of GBS meningitis was 26.3 % in infants under 2 months of age. This study have noticed the emergence of GBS as a main cause of neonatal meningitis in Malawi and sub-Saharan Africa region (22) . Similarly in South Africa, GBS caused more than 44% (32/72) of clinically diagnosed meningitis cases. Based on this study finding; South Africa had encountered an 18.0% case fatality rate of invasive GBS disease in infants < 3months of age (34).

In Ethiopia, many studies have been focused on maternal GBS colonization (18-20) and has shown a variable rate of colonization, from 14.6% (19) to 19% (20). However, only few articles demonstrated colonization rate of GBS in the newborns with 8.9% in Hawassa (18) and 16.1% in Gondar (55). In contrast, the 2011 EDHS and 2015 data report has shown meningitis among the top ten causes of death in infants. According to that report, meningitis was the substantial causes of post neonatal and child mortalities among infectious diseases (56).

Study on magnitude of BM, especially GBS in infants, is unavailable. A single retrospective study (2001- 2010) in TASH has shown a 4.7% prevalence of bacterial meningitis among suspected neonates. The most common pathogens identified in the study were *S. pneumoniae* (23 %) and *E. coli* (16 %) (23). However, there is unavailability of data that mentions GBS role in meningitis in Ethiopia, as well as there is no local epidemiology for prevention strategies. These inputs directed our focus to this issue.

2.6.3. Pathogenesis of GBS

Bacterial meningitis is usually preceded by nasopharyngeal or middle ear colonization, followed by microbial invasion of the tissue and intravascular space and bacteremia. The guarantee of many bacteria that infected the CNS is their survival ability in the blood stream either by avoiding or protecting against phagocytosis. Meningeal invasion occurs following penetration of the cellular barriers blood-brain barrier (BBB) (57). The blood-borne GBS must typically penetrate the BBB to produce meningitis. Therefore, disruption of BBB integrity is a hallmark occurrence in the pathophysiology of BM. The BBB disruption could resulted as consequence of synergetic effect of bacterial entry and penetration of brain micro-vascular endothelial cells (BMECs), direct cellular injury by bacterial cytotoxins, and/or activation of host inflammatory pathways that compromise BMEC barrier function(5, 58). Specifically the cytolytic toxins of GBS can damage host cells thereby leading to disruption of the barrier and mediation of para-cellular invasion (5). When the pathogen reached to the brain, bacteria (or bacterial components) are recognized by resident immune cells (such as microglia and astrocytes) leading for their activation. Then after, circulating professional immune cells, such as granulocytes and monocytes/ macrophages would be attracted and subsequently infiltrate the infected brain parenchyma. The resulting antibacterial immune response might be devastating, if hosts are neonates, leading to a pronounced neuronal damage and even death (5).

2.7. Role of cfb gene as a diagnostic tool/ marker

The CAMP factor, the extra-cellular protein produced by GBS was used as a presumptive diagnostic test for confirming GBS and GBS like colonies developed on blood agars (13, 49). This is due to the availability of CAMP factor in almost all clinical GBS isolates. Accordingly, the gene responsible for coding the CAMP factor and present in the vast majority of GBS isolates (cfb) were utilized for the molecular identification of GBS (49). Based on published literatures first PCR developed for GBS screening purposes was targeting the CAMP factor coding gene and this assay is known to provide promising results besides its rapidity in detection and identification of GBS(59).

After the first PCR developed for GBS onwards, studies has produced different interesting finding particularly on samples negative with gold standard method/microbiologic culture

methods. Tests targeting cfb gene can both confirm the presence of *S. agalactiae* bacteria as well as depict the most determinant virulent factor/CAMP (60). This could also be justified through routinely used bacteriological biochemical tests as the suspected colonies were identified as GBS by catalase test, Bile-Esculin test and confirmed as GBS by CAMP test (8).

Recent studies also show the clinical implications of this advanced technique because of its sensitivity and specificity, beside its rapidity. According to the study done in Brazilian, there were a better performance of cfb gene targeted PCR compared to culture method, with a sensitivity and specificity was 100% and 85.6% respectively and suggest its suitability for routine screening (61).

Group B streptococci colonization in vaginal and rectal specimens were examined by culture method and PCR technique in Iran and has shown 42/137 (30.7%) rectal and 38 /137 (27.7%) vaginal positivity via culture in contrast to 57/137(43.8%) rectal and 60/137 (43.8%) vaginal detection rate by PCR which amplifies the cfb gene (62). Similarly 50 pregnant women were screened for vaginal GBS colonization in India. Of them 16%(8/50) were positive through culture, while 62% (31/50) by PCR targeting the cfb gene, offers 46% more detection performance than culture method (8).

Many diagnostic guidelines still recommend the use CAMP test as the key confirmatory test for identification of GBS, it lacks sensitivity. The existence of CAMP negative GBS strains controversies its role in confirmation. However, the gene responsible for coding CAMP are of worth to customize as diagnostic tool since this target gene is available in all GBS strains including CAMP negatives (63). The study in china has shown that culture negative sample could result in positive if tested with more sensitive tool like PCR. In this study 50.7% positivity was observed when specific gene targeted PCR was conducted (64). Therefore, it is too critical to consider such diagnostic tool since we found enhanced results.

3. OBJECTIVES

3.1. General objective

The aim of this study was to determine the magnitude and its associated factors of Group B Streptococcus in culture negative infants suspected of meningitis at TASH from June to October 2018

3.2. Specific objectives

1. To determine the magnitude of Group B Streptococcus in culture negative infants suspected of meningitis through PCR
2. To assess commonly associated socio-demographic and clinical factors with Group B Streptococcus

4. MATERIALS AND METHODS

4.1. Study area and period

The study was conducted at Tikur Anbessa Specialized Hospital (TASH), Addis Ababa, in collaboration with AHRI. It is estimated to offer diagnostic and therapeutic services for more than half a million patients per year including over 520 meningitis cases. Within this study setting, the magnitude of *S. agalactiae* was assessed in infants with suspected meningitis from June to October 2018.

4.2. Study design

Institution based cross-sectional study was conducted at TASH, Addis Ababa from June to October 2018.

4.3. Study population

Routinely, infant patients suspected clinically for meningitis were undergone lumber puncture (LP) with possible aseptic procedure. CSF samples were sent to lab for white cell count, protein and glucose measurement, gram staining and culture within one hour of collection. The target population in this study was those infants under 1 years of age and of both gender from Tikur Anbessa specialized hospital who were clinically suspected with meningitis (infants with sudden onset of fever, meningeal irritation or altered consciousness) from June to October 2018.

4.4. Sampling technique and sample size determination

All the infants under 1 year with clinically suspected meningitis were included conveniently for the study. Therefore a total of 72 infants aged from one day to one year were included. The sample size determination was calculated based on single population proportion as follows.

$n = \underline{((Z\alpha/2)^2 \text{ x P } (1 - P))/D^2} = \underline{(1.96)2\ (0.047)\ (0.953)/0.0025} = 69$ adding 10% non-response rate sample size was 76.

- Z = value from standard normal distribution corresponding to desired confidence level (Z=1.96 for 95% CI),
- P is proportion of neonates with bacterial pathogens isolated at TASH (23) and
- D is desired precision/margin of error. Therefore, to draw conclusions we need 76 infants with suspected meningitis.

4.5. Inclusion and exclusion criteria

For this study infants age less than or equal to one year and with parents' consent granted were included. In addition, CSF sample volume above 200ul and parents' consent grant, those with incomplete data such as CSF white cells count, protein, glucose and patients outcome (as this was from secondary sources) were also included in the study. While, infants with CSF sample volume less than 200ul were excluded from enrollment.

4.6. Laboratory procedure

During the study period a total of 76 study participants found for enrollment. However, 3 CSF samples were excluded from the study due to insufficient amount. Two CSF samples were from one infant (this infant was expected to progress after the first tap, meanwhile the infant condition was not recovered and re-collected) and we considered as one. Seventy two CSF samples, collected under aseptic conditions were submitted to microbiology laboratory of TASH and processed within 1 hour after collection (65). The macroscopic appearances of the samples were noted as clear, turbid yellow or bloody. Similarly CSF parameters (protein using mindray (Mindray, Shenzhen-China) CSF mode, while glucose was measured like serum glucose) and white cells count using sysmex body fluid mode and Gram staining was done on the CSF sediments found after centrifugation at 1000g for 10 to 15 minutes. Subsequently the CSF was cultured onto brain heart infusion (BHI) broth, chocolate and blood agar plates (both incubated with 5% CO_2) and onto MacConkey (in aerobic condition), then incubated at 35-37°C range for up to 72hrs. The BHI broth was incubated under shaker incubator at 37°C for up to 72hrs so as facilitate microbial growth in aerobic condition. The remaining CSF samples were used to extract the genomic DNA using Qiagen DNA mini kit (QIAamp DNA mini kit (250), Hilden, Germany) according to manufacturer's instruction (see **Annex2**). The mean DNA concentration

was 21.3 ng/ul (minimum 5.8 ng/ul to maximum 53.9 ng/ul) and the purity was in a range of 1.7 to 2.0 with bio- spectrometer (Eppendorf AG, Hamburg, Germany). GBS specific primer used were targeting the gene encoding CAMP factor (*cfb*) (Sequence ID: MK134700.1) for specific detection of GBS, since CAMP factor is a major virulence determinant in GBS among most serotypes. The reaction mixtures (25µl) contained 12.5 µl HotStarTaq mix, 1.5 µl (5uM) of each of SAG 59 (TTTCACCAGCTGTATTAGAAGTA) and SAG 190 (GTTCCCTGAACATTATCTTTGAT) primers, 4.5 µl molecular grade water, and 5 µl DNA template and with an initial denaturation at 94°C for 12 minutes, followed by 40 cycles of amplification (denaturation at 94°C for 30 seconds, primer annealing at 55°C for 30 seconds and extension at 72°C for 30 seconds). A final extension step was carried out at 72°C for 2 minutes.

Gel electrophoresis was run on 2.0 % agarose gel for PCR amplified products to visualize the target bands with 100bp DNA ladder as the reference marker and DNA of GBS (ATCC 12386) as positive control with 153bp amplicon size. 2.0 % agarose gel was briefly prepared by measuring 1.0 gram of agarose into 50 ml of 1x TAE (Tris Acetate EDTA (Ethylene-Diamine-Tetra Acetic-acid)) buffer and melted with microwave oven for 1:30 minutes at 900 watts. When the agarose solution was cooled to hand touch (nearly 50°C), 3 ul of ethidium bromide (10mg/ml) was added into 50ml of prepared gel. Once the amplification process has finished, PCR product was loaded onto the gel using loading dye in a proportion of 1 part of loading dye into 5 parts of amplified sample to load.

4.7. Study variables

4.7.1. Dependent variables

Group B streptococcus in under 1 year infants

4.7.2. Independent variables

Socio-demographic and clinical characteristics

- Age
- Sex
- Sign and symptoms (fever, neck stiffness, altered consciousness , vomiting)

- CSF parameters (White cell count/ul, CSF protein and glucose level)
- Antibiotic usage before spinal tap
- Outcome of patients (recovery or death).
- CSF macroscopic observation/ appearance

4.8. Data collection procedures (Instrument, personnel, data quality control)

The routinely suspected cerebrospinal fluid (CSF) samples sent to TASH microbiology lab for culture and the assigned lab staff has noted appearance of CSF and its culture result with the case record form. Then for infants with granted parents' consent, the socio-demographic profiles, clinical presentations and CSF parameters (i.e., white cells count, protein and glucose concentration) were reviewed from patient's medical record (as a secondary source) in their inpatients wards by the trained nurses using a case report form as a data collection tool, from June to October, 2018.

4.9. Data processing and analysis

Data was checked and cleared for incompleteness, and then entered into Epi-data version 3.1. Moreover, cross-checking and data cleaning were done, and finally got transferred to SPSS version 25.0 for analysis. Chi-square ($\chi 2$) test was used to determine the association between variables and compare level of association with the outcome variable. Binary logistic regression was utilized for fitness if $p< 0.25$ and multivariate logistic regression was performed to test for association with meningitis. The $p <0.05$ was considered to represent a statistically significant for association.

4.10. Data quality management

Technical updates were provided to data collectors what specific information's to compile and communicate with infant's parent for consent. Available secondary data such as CSF white cells count, protein and glucose concentration were used, while for those with no such available data were included as no result. Training was provided to principal investigator on basic PCR operation and analysis system by AHRI experts. DNA from ATCC 12386, positive control (suspended and cultured on nutrient broth and extract DNA from broth culture at extraction room)

was extracted for protocol optimization and the optimized protocol works well in multiple runs for ATCC DNA and molecular grade water (negative control). Before and after each master mix preparation procedures, the biosafety cabinet was cleaned with 10% bleach and with 70% alcohol to prevent any contamination. The rooms for master mix preparation and place for template addition were different rooms and sample storage room as well. The data generated was checked for completeness and double entered to minimize errors. Furthermore appropriate, positive (*streptococcus agalactiae*, ATCC 12386 was supplied by EPHI) and negative (molecular grade water) quality controls were utilized to assure the quality of data we generate.

4.11. Ethical consideration

Before sample collection, an ethical clearance was obtained from AHRI/ALERT ethics review committee with protocol number PO 14/18 and from an institutional review board of Jimma University with a reference number IHRPGD/143/2018. Moreover, a support letter was also obtained from Jimma University, school of medical laboratory sciences before conducting the study and written informed consent was obtained from infants parents or guardian for use their infants leftover CSF to this study. Study participants right to refuse and not give CSF sample without affecting their routine medical services were granted. Findings were immediately communicated with pediatricians at TASH so as to help infants if still admitted and to take into consideration result on which segment of infants were burdened with GBS.

4.12. Operational definitions in this study

GBS meningitis =presence of GBS in CSF in association with a clinical diagnosis of meningitis

Early onset disease (EOD) = infants aged 0-6 days

Late onset disease (LOD) = infants aged 7-89 days

Ultra late onset disease (ULOD) =infants beyond 3 months of age

Fever = temperature beyond 38^0c either axillary or rectal

Infants = study participants with age less than one year

Altered consciousness = a spectrum of disease ranging from mild agitation to coma, impaired consciousness is alternatively used here for altered consciousness (66)

4.13. Dissemination of the Results

The findings of this research was presented in a form of thesis and submitted to Jimma University School of Medical Laboratory Sciences and AHRI. The findings will also be presented on conference stages of national and/or international level. Furthermore, manuscript will be prepared and submitted for publication on peer-reviewed scientific journals.

5. RESULTS

Out of the 72 infants enrolled to this study, the proportion of infants with their age; 61.1 % (n=44) were neonates (aged ≤ 28 days) and the remaining 38.9 % (n=28) of them were in age beyond neonatal period. The mean age at onset of meningitis was 49.8 ± 83.6 days (1-365 days). The male to female proportion was nearly 1.7:1 i.e. 62.5% being male infants.

Fever and headache were the two early clinical events expected to occur in most cases of meningitis. In our study, 43% (n=31) of total infants were observed to have fever. Overall, vomiting or reduced ability to suck breast feeding (n=45), fever (n=31) and altered consciousness (n=27) were the most frequent clinical presentations noted in our study. Of the clinical presentations examined; 61.3% (n=19) of infants with fever, 48.1% (n=13) with impaired consciousness and 55.6% (n=25) of infants who had vomiting or reduced ability to suck for breastfeeding were positive for GBS (table 1). Most of the infants (75% (n=54)) had a history of antibiotics use prior to spinal tap procedure, which could decrease GBS culture recoverability.

According to the clinical disease onset by age, most of the infants were classified under late onset of disease (LOD) as observed by the proportion of 76.4 % (55/72). While the proportion of early onset of disease (EOD) and ultra-late onset of disease (ULOD) were 12.5% (9/72) and 11.1% (8/72) respectively as shown below in figure.1.

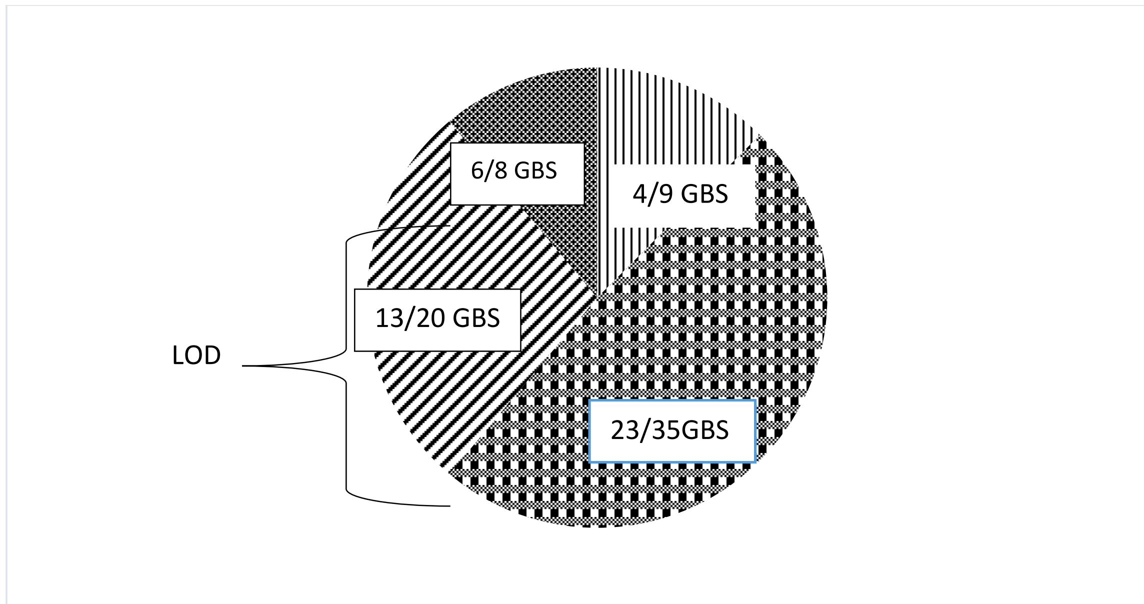


Figure 1 : proportion of infants stratified with age that is PCR positive for GBS out of total infants enrolled in the study.

The CSF white cells count, protein and glucose concentrations were obtained for more than half of the infants. Unlike most criteria's used to exclude bacterial meningitis, GBS was detected in more than 67.4% (n=29) of infants without pleocytosis (as defined by CSF cells count of ≥ 20 cells /µl) in our study. Of the infants with available glucose concentration data, 8(out of 35) had below 40 mg/dl, while from infants with protein concentration data 16(out of 46) had above 100mg/dl, as literally expected in bacterial meningitis cases.

Similarly the appearance of CSF was noted and nearly 78% of all the samples were found clear. Most of the confirmed GBS cases in our study has presented normal CSF parameters in terms of cells count, protein and glucose concentrations (see table 1). This could question the reliability of CSF parameters in the prognosis of BM.

The relatively higher number of male study participant's incorporation in the study, makes the proportion of GBS to be higher among male study participants (66.7% (30/45) than females (59.3% (16/27) (fig.2.).

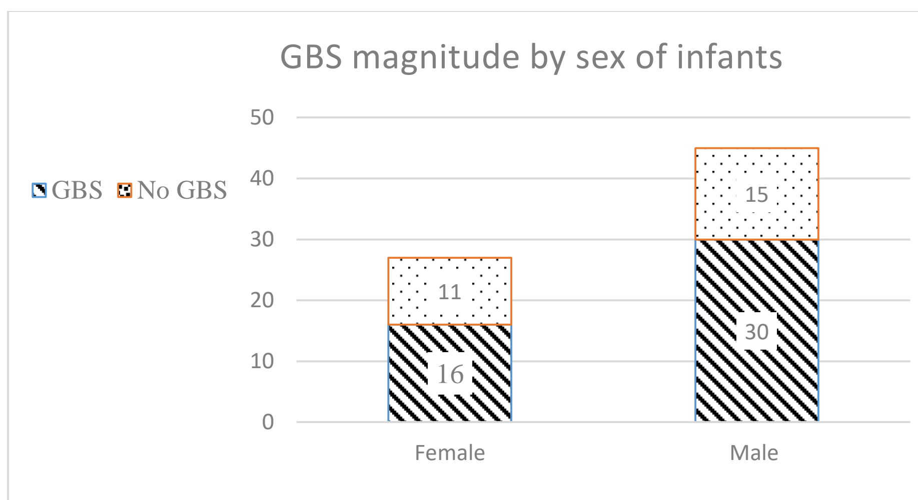


Figure 2 : GBS magnitude among infants under the age of less than one year old against gender at TASH from June. 2018 to Oct. 2018, Addis Ababa, Ethiopia.

The overall magnitude of GBS in infants with suspected meningitis was 63.9% (46/72) (fig.3). High prevalence of GBS was noted in young infants above 7 days of age, given above 65.5% of GBS positives were infants beyond 7 days (table 1).

Table 1: Bivariate analysis of demographic and clinical features of infants suspected with meningitis at TASH from June 2018 to Oct. 2019, Addis Ababa, Ethiopia.

Characteristics		GBS		COR (95%CI)	p-value
		Positive (%)	Negative (%)		
Age	< 7 days (n=9)	4 (44.4)	5 (59.6)	0.27 (0.03,2.12)	0.211
	7 -89 days (n=55)	36 (65.5)	19 (34.5)	0.63 (0.12,3.44)	0.595
	≥ 90 days (n=8)	6 (75)	2(25)	R	
Sex	Male (n=45)	30(66.7)	15(33.3)	1.375 (0.51 ,3.69)	0.526
	Female(n=27)	16(59.7)	11(40.3)	R	
Fever*	Yes (n=31)	19(61.3)	12(38.7)	0.82(0.31,2.16)	0.690
	No (n=41)	27(65.9)	14(34.1)	R	
Impaired consciousness	Yes (n=27)	13(48.1)	14(51.9)	0.34(0.12,0.92)	**0.034**
	No (n=45)	12(26.7)	33(73.3)	R	
Vomiting/reduced breastfeeding	Yes (n=45)	25(55.6)	20(44.4)	0.36(0.12,1.05)	**0.062**
	No(n=27)	21(77.8)	6(22.2)	R	
Neck stiffness	Yes(n=2)	1(50)	1(50)	0.56(0.03,9.27)	0.682
	No(n=70)	45(64.3)	25(35.7)	R	

CSF WCC^	<20 cells/µl (n=43)	29(67.4)	14(32.6)	R	
	≥ 20 cells/µl (n=11)	8(72.7)	3(27.3)	1.29(0.29, 5.61)	0.737
CSF glucose^	≤ 40mg/dl (n=8)	6(75)	2(25)	1.26(0.21,7.65)	0.799
	> 40mg/dl (n=27)	19(70.4)	8(29.6)	R	
CSF protein ^	≤ 100mg/dl (n=30)	20(66.7)	10(33.3)	R	
	> 100mg/dl (n=16)	10(62.5)	6(37.5)	0.83(0.24, 2.96)	0.778
Outcome of infants^	Recovery (n=16)	5(31.3)	11(68.7)	R	
	Death (n=6)	5(83.3)	1(16.7)	11.0(1.005,120.43)	**0.050**

** Fever = temp. ≥ 38 ^{o} c, unknown/no result =data not available on the patient's medical record.*

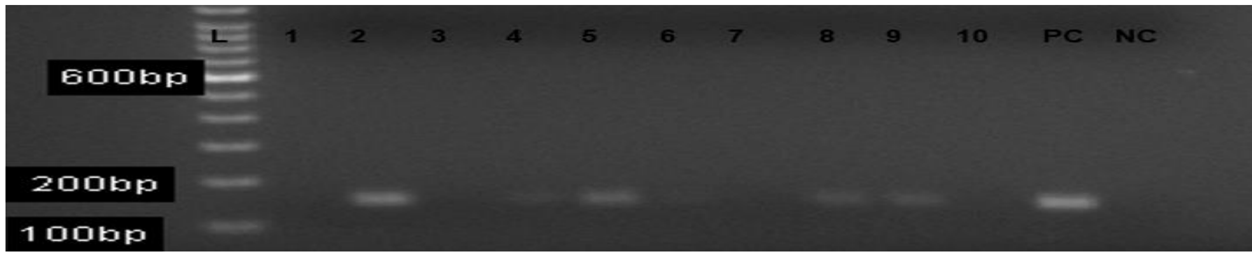
** Normal values: WCC= 0-5/µL, CSF Protein =40-100 mg/dl and CSF glucose =40-75 mg/dl*

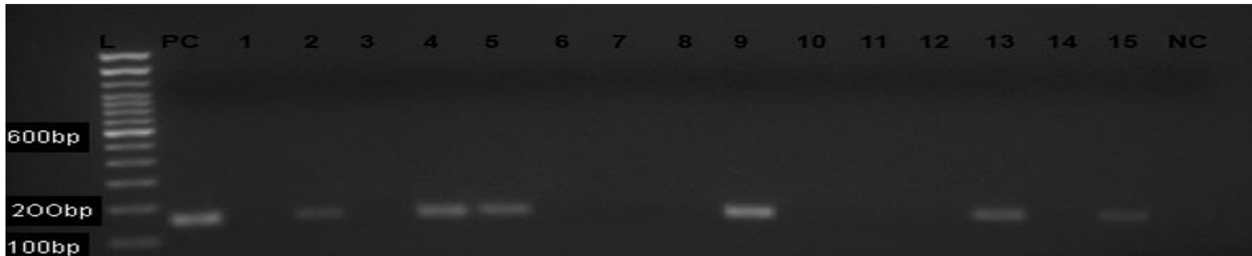
** Bold p-values: variables tested fit for multivariate analysis after bivariate checkup (some of the associated factors such as age, impaired consciousness, vomiting, and CSF white cells count and patients outcome were among variables fit for multivariate logistic regression).*

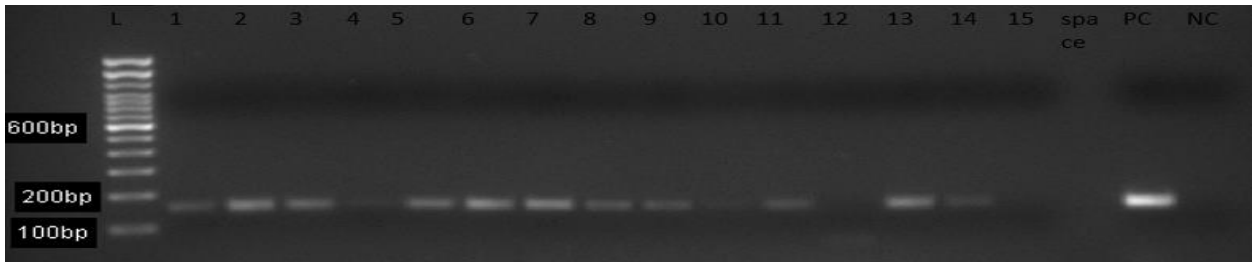
^ done with available data since this used secondary data from patients medical record.

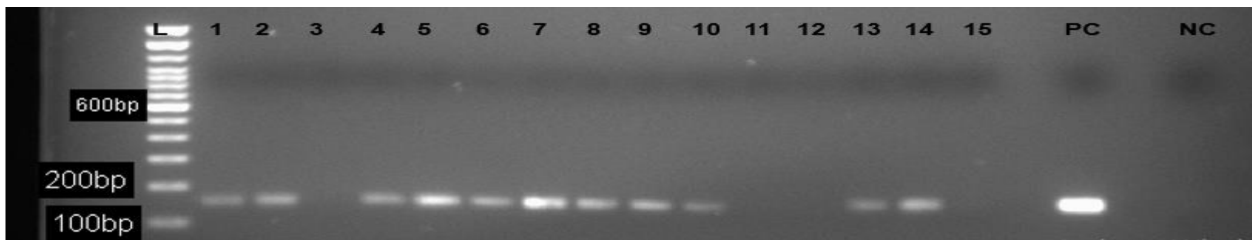
Death was observed in 6 of the 72 enrolled study infants. Death might be attributed by GBS in 10.9% (5out of 46 confirmed GBS cases) meningitis cases. Four of the died infants were in age group less than or equal to 28 days, while one was 44 days old and the other one was 4 months old. Considering the rate of GBS positivity (GBS vs. no GBS), the probable death was assumed (10.9% (5/46) vs. 3.8 %(1/26)).

Based on the binary logistic regression result, clinical presentations such as impaired consciousness and outcome of patients (full recovery, death or unknown status due to transferred out, missed with their appointment, parent withdrawal against medical advice) has shown to have a statistically significant association with GBS (p <0.05)(see table 1.).









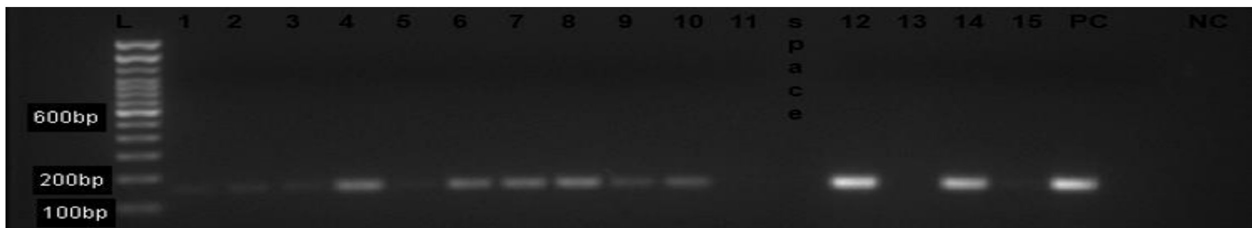


Figure 3: photo 1-5(day 1-5) are PCR reading with gel doc system for GBS using 100bp DNA ladder with an expected amplicon size of 153bp. L was the reference ladder. # 1-15 were patient samples, PC was positive control, while NC was negative control.

6. DISCUSSION

In the present study, GBS was found high in infants with suspected meningitis cases, 63.9 % (46/72), which is quite comparable with study done in Korea 69 % (53) and lower to US 86.1% (51). However, it was higher relative to study results in China (46.5%) (44), in USA (53.6 %) (26) , in England (52 %) (50), in South Africa (44%) (34) and in Malawi (26.3 %) (22). The difference observed could be the detection method utilized, maternal use of IAP, study setting differences or population variations. Possibly inclusion of infants' age beyond 3 months and the use of more sensitive detection tool in our study may increase proportion of GBS detection.

Detection through cfb gene targeted PCR was found too enhanced from all culture negative results of GBS to 63.9 %. Similar studies have demonstrated the positivity of culture negative CSF samples, when tested with more sensitive techniques like PCR. Culture finding could be negative, possibly due to previous antibiotic exposure prior to CSF collection, low number of bacterium, lower inoculum size, or absence of centrifugations before inoculation. A comparative study in Ireland has shown the reputation CSF PCR. Considering culture as a gold standard tool, 77.3 % more detection capacity was offered by PCR (67). Other studies in Brazil (3.8 % vs. 29.2 %) (61), Iran (27.7 % vs. 43.8 %) (62), China (0 % vs. 50.7 %) (64) and Jimma, Ethiopia (3 % vs. 33 %) are indicating the presence of more bacterial pathogens as detected through PCR (21).

Late onset GBS disease (65.5 %) was observed high relative to EOGBS (44.4%) (Table 1). However, this study finding was relatively lower compared to results in China (74 %) (68) and in US (86.5 %) (51). High community or nosocomial acquisition, exposures to colonized parents and siblings, breastfeeding during maternal mastitis and or prematurity could be the possible reasons (25, 69). Although our sample size was too small, infants presented with LOGBS was more likely to have death outcome, i.e. 3 out of 5 GBS positive deaths were LOGBS. Similar studies have shown that the LOGBS causes higher morbidity and mortality than EOGBS and ULOGBS at the time of discharge (25, 69). Australian finding has verified infants with LOD case were 3 times more likely to develop neurodevelopmental sequelae or die in the course of admittance than ULOD, this reflects the vulnerability of infants in the first 90 days of life (25).

Disease presentations such as fever and headache were the two early clinical events to occur in most cases of meningitis (70). This study noted vomiting or reduced ability to suck breast feeding (n=45), followed by fever (n=31) and altered consciousness (n=27) as the most frequent clinical presentations. Cerebrospinal fluid parameters such as protein, glucose, and white cell count (WCC) were used to assess the likely etiological agents, provide a protein concentration <60 mg/dl and WCC <90 cells/ µl were found to be optimal cut-offs for excluding bacterial meningitis (47). Opposing this conclusion, the present study found more than 67 % of infants positive for GBS had WCC < 20 cells/ µl. Similar study in India has also described 16 % of infants with GBS had 0-20 cells/ul (35), and in USA nearly 11 % of cases lacked CSF pleocytosis (26). Other similar studies has also shown the occurrence of bacterial meningitis without pleocytosis, in a normal CSF (3, 45).

Due to the difference in study settings, socio-economic status and living standards, comparing such findings with US and Australia may not be fair. On other hand, our results may overestimated the morbidity and mortality of GBS meningitis, since all of the infants were from the largest tertiary and referral hospital with more severe illness than seen in regional centers elsewhere in the country. The probable mortality of GBS meningitis was 10.9 % (5/46) in present study, lower compared with study result in South Africa (18 %) (34), due to the inclusion of all the invasive GBS infections (including sepsis and meningitis) in South Africa. The use of limited sample size, unavailability of data of infants who are transferred out, lost with their appointment and withdraw against medical advice, inclusion of infants with access of health facilities may hinder the real burden of mortality in the present study.

Limitation of the study

This study tried to address magnitude of GBS meningitis in infants and gave an insight how more sensitive tools would implicate for culture negative suspected meningitis cases. However, the main constraint to this study was the use of limited sample size and inability of our study to address potential risk factors such as mode of delivery, maternal colonization,

maternal mastitis, birth gestational age and birth weight as this study used secondary data regarded factors were not available.

CONCLUSION AND RECOMMENDATIONS

Group B streptococcus (GBS) was found the major etiologic agent of BM among neonates and young infants, given that high magnitude of GBS was detected in CSF samples of 72 infants suspected with meningitis at TASH, Addis Ababa, Ethiopia from June to October 2018. The current study showed that the onset of disease matters to the outcome or progress of patients, indicating that three out of five died infants had late onset presentation. The role of GBS as an etiologic agent of BM was underestimated by culture detection techniques in contrast to PCR, since this study found that 63.9% of infants to have GBS whom were culture negative, and most of them had history of antibiotic exposure before spinal tap.

Health care professional, health policy makers and other concerned bodies need to be informed about the potential role of GBS in meningitis in neonates and young infants. With this regard, incorporation of GBS in the list of potential bacterial pathogens causing meningitis is recommended. Hence, cfb gene targeted PCR were shown to have better GBS detection capacity; concerned body may take into consideration for at least to the referral hospitals. Further studies of this kind with potential risk factors and larger sample size needs to be conducted so as to devise basic prevention and control strategies.

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ANNEXES

Annex I: Oligonucleotide sequence primers used for PCR detection of GBS

Target gene	Oligonucleotides	sequences (5 - 3`)	Length	Amplicon size (bp)
Cfb	Sag 59	TTTCACCAGCTGTATTAGAAGTA	23	153
	Sag190	GTTCCCTGAACATTATCTTTGAT	23	

Annex II: DNA extraction protocol from body fluids (using Spin Protocol)

This protocol is for purification of total (genomic, mitochondrial, and viral) DNA from whole blood, plasma, serum, buffy coat, lymphocytes, and body fluids using a microcentrifuge. All centrifugation steps are carried out at room temperature (15–25°C) and 200 µl of whole blood yields 3–12 µg of DNA.

1. Pipet 20 µl QIAGEN Protease (or proteinase K) into the bottom of a 1.5 ml microcentrifuge tube.
2. Add 200 µl sample to the microcentrifuge tube.
3. Add 200 µl Buffer AL to the sample. Mix by pulse-vortexing for 15 s.
4. Incubate at 56°C for 10 min**.** DNA yield reaches a maximum after lysis for 10 min at 56°C. Longer incubation times have no effect on yield or quality of the purified DNA
5. Briefly centrifuge the 1.5 ml microcentrifuge tube to remove drops from the inside of the lid.
6. Add 200 µl ethanol (96–100%) to the sample, and mix again by pulse-vortexing for 15 s. After mixing, briefly centrifuge the 1.5 ml tube to remove drops from the inside of the lid.
7. Carefully apply the mixture from step 6 to the QIAamp Mini spin column (in a 2 ml collection tube) without wetting the rim. Close the cap, and centrifuge at 6000 x *g* (8000 rpm) for 1 min. Place the QIAamp Mini spin column in a clean 2 ml collection tube (provided), and discard the tube containing the filtrate.
8. Carefully open the QIAamp Mini spin column and add 500 µl Buffer AW1 without wetting the rim. Close the cap and centrifuge at 6000 x *g* (8000 rpm) for 1 min. Place the QIAamp Mini spin column in a clean 2 ml collection tube (provided), and discard the collection tube with the filtrate.
9. Carefully open the QIAamp Mini spin column and add 500 µl Buffer AW2 without wetting the rim. Close the cap and centrifuge at full speed (20,000 x *g*; 14,000 rpm) for 3 min.
10. Recommended: Place the QIAamp Mini spin column in a new 2 ml collection tube (not provided) and discard the old collection tube with the filtrate. Centrifuge at full speed for 1 min.
11. Place the QIAamp Mini spin column in a clean 1.5 ml microcentrifuge tube (not provided), and discard the collection tube containing the filtrate. Carefully open the QIAamp Mini spin column and add 200 µl Buffer AE or distilled water. Incubate at room temperature (15–25°C) for 1 min, and then centrifuge at 6000 x *g* (8000 rpm) for 1 min.

Annex III: informed consent (English version)

Jimma University

School of medical laboratory sciences

Department of medical microbiology

Consent form prepared to assess the magnitude of GBS in infants with suspected meningitis at Tikur Anbessa specialized hospital, Addis Ababa, Ethiopia.

Code (continuous from 001): _____________________

I hereby undersigned to take part in the study to be done at AHRI in collaboration with Jimma University School of medical laboratory sciences, Department of medical microbiology. I have informed the objective of the study “Enhanced identification of GBS in infants with suspected meningitis at TASH, Addis Ababa, Ethiopia” which is very essential to rule in GBS responsible for meningitis in our country. The study findings importance were briefed to me, as clinicians may use to improve meningitis management and could inform government on GBS meningitis that can be preventable with parental vaccine. It is also described that the name of participant will not be written in the form and was kept strictly confidential. My participation was voluntarily and is not obliged to answer any question that i do not want to answer. If i fill discomfort with the interview, am free to drop it any time i want. Having understood, I have decided to participate in this study. It is, therefore, with full understanding of the situation that I gave the informed consent voluntarily to the researcher to use my child CSF sample for the intended research.

Agree_______ Not agree________

Signature of participant ______________________Date________

Interviewer signature _______________ date __________

Site of data collection ________________________

Annex IV: informed consent (Amharic version)

በ ጅማ ዩኒቨርሲቲ እና አርማዉር ሐንሰን የምርምር ተቀም ተዘጋጀ የስምምነት ውል- አማርኛ ግልባጭ

ይህ የስምመነት ቅፅ የተዘጋጀው ዕርስዎ ተሳታፊ እንዲሆኑ ለተጋበዙበት ምርምር ቡድኑ የሚያካሂደውን ጥናት በተመለከተ የዕርስዎን ፈቃደኝነት ለማወቅ ነው፡፡ የምርምር ፕሮጀክቱ ዋና በጥቁር አንበሳ ሆስፒታል ለማጅራት ገትር የታከሙ ህሙማኖችናሙና ላይ ምክንያት የመጣውን ማጅራት ገትር ማወቅና መለየት ነው፡፡

እኔ ከዚህ በታች ፊርሚዬን ያኖርኩት የጥናቱ ተሳታፊ "በጥቁር አንበሳ ዩኒቨርሲቲ ሆስፒታል ለማጅራት ገትር የታከሙ ህሙማኖች ናሙና ላይ አምጭውን ባክተርያን መመርመር " በሚል አርዕስት ሊጠና በታሰበው ምርምር ላይ በቂ መረጃ ያገኘሁ ሲሆን የጥናቱ ዓላማም ሆነ የጥናቱ ዘዴ እኔ በምሰማዉ ቋንቋ ስለገለጹልኝ ተረድቻለሁ፡፡ ሌላዉ ጠቀሜታ ማለትም ማጅራት ገትርን በማከም ደረጃ ፤ ብሎም ለመንግስት መረጃ ለመስጠት እንደሚያስችል ገለጻ ተደርጎልኛል፡፡ በተጨማሪም በጥናቱ ላይ ስሜ የማይገለጽ ሲሆን ፤ የሚወሰዱ ማናቸውም መረጃዎች በሚስጢር እንደሚያዙ እና የምጠየቀውን መረጃ ያለመስጠትና በጥናቱ ያለመሳተፍ ሙሉ መብት እንዳለኝ እንዲሁም ከጥናቱ በማናቸውም ጊዜ ራሴን ማግለል እንደምችል የተገለፀልኝ ሲሆን የጥናቱን አጠቃላይ አላማ በመረዳት ይህን የስምምነት ውል በፈቃደኝነት በመፈረም ለጥናቱ የሚያስፈልጉ መረጃዎችን እና በናሙናዉ ላይ የታሰበዉ ጥናት እንዲሰራ ተስማምቻለሁ፡፡

የተሳታፊው ፊርማ________________ቀን____________

መረጃዉን የሚሰበስበዉ ሰዉ ፊርማ____________ ቀን____________

መረጃ የተሰበሰበበት ቦታ __________________________

ምርምሩን የሚካሄደዉ ሰዉ ፊርማ____________ ቀን ________

የታዛቢዎች ፊርማ:1. ____________ ቀን ________

2. _____________ ቀን ________

3. _____________ ቀን ________

Annex V: Information sheet (English version)

Title of the study: Enhanced identification of GBS in infants with suspected meningitis at TASH, Addis Ababa, Ethiopia.

Objective of the study: To detect GBS in infants with suspected meningitis at Tikur Anbessa hospital, Addis Ababa, Ethiopia.

Introduction: This information sheet is prepared to explain the study you are asked to join. Please listen carefully and ask any questions about the study before you agreed to take part in. This interview may take nearly 10 minutes and I request your patience please.

Procedure: You are invited to join this study because the burden of GBS meningitis in infants is unknown. If you are willing to participate in this study, you will help us to identify GBS as cause of meningitis. So you need to understand and sign the agreement form for the leftover samples to use for this study. Your privacy kept confidentially by using coding system whereby no one will have access to your information.

Risk of the study: The study has a minimal risk to you as the study uses the already collected samples. **Benefit of the study:** Your participation benefits you through identifying the etiology of meningitis for your better clinical care. The findings of this study will also generate evidence to the health policy makers to make informed decision and better clinical care for other patients with meningitis. **Confidentiality**: The information collected from this study kept confidential and information generated in this study will be stored as a file with a code assigned. **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, without losing any of your right.

Persons to contact: For study related questions, please contact the following individuals:

Alene Geteneh (Study PI), phone: +251-932-47-03-27 / Dr. Wude Mihret (study supervisor), phone: +251-910-49-86-41

For ethics related questions, please contact the AHRI/ALERT Ethics Review Committee secretariat at, 215-113 481289

Annex VI: Information sheet (Amharic version)

በ ጅማ ዩኒቨርሲቲ እና አርማዉር ሐንሰን የምርምር ተቀም ተዘጋጀ የጥናቱ መረጃ መስጫ፤ አማርኛ ግልባጭ

የጥናቱ ርዕስ - በጥቁር አንበሳ ዩኒቨርሲቲ ሆስፒታል የማጅራት ገትር ቫክርያ ልየታ

የጥናቱ አላማ- በጥቁር አንበሳ ሆስፒታል ለማጅራት ገትር የታከሙ ህሙማኖች ናሙና ላይ የቫክርያውን ምክንያት መለየት

መግቢያ-ይህ የመረጃና የስምመነት ቅፅ የተዘጋጀው ዕርስዎ ተሳታፊ እንዲሆኑ ለተጋበዙበት በምርምር ቡድኑ የሚያካሂደውን ጥናት በተመለከተ የዕርስዎን ፈቃደኝነት ለማወቅ ነው። የምርምር ፕሮጀክቱ ዋና ፍላጎት በጥቁር አንበሳ ዩኒቨርሲቲ ሆስፒታል ለማጅራት ገትር የታከሙ ህሙማኖች ናሙና ላይ የቫክርያውን ምክንያት ለማወቅና መለየት ነው።

የጥናቱ ዘዴ- እርሰዎ በዚህ ጥናት የተጋበዙበት ምክንያት GBS የተባለው ቫክርያ ማጅራት ገትር ተያዙ በተባለው ልጅ ላይ ጥናት የለም ። ስለሆነም ፈቃደኛ ከሆኑ ይህንን የስምምነት ፎርም ይፈርማሉ። እናም የህመምዎን አምጭ ተህዋስ ለማወቅ በተወሰደው ናሙና ላይ ጥናት ይደረጋል።

የትናቱ ጉዳት-ተሳታፊው በዚህ ጥናት ውስጥ በመሳተፋቸው የሚደርስባቸው ምንም አይነት ጉዳት የለም፤ ምክንያቱም ጥናቱ ከህክምናዎት የተረፈዉን ናሙና ስለሚጠቀም ነዉ።

የጥናቱ ጥቅም- በዚህ ጥናት ተሳታፊ በመሆንህ GBS ወለድ ማጅራት ገትር መኖር አለመኖሩን ምርመራ ይደረግልሃል፤ ለተሻለ እንክብካቤም ይጠቅምሃል። ጥናቱ ለሌሎች ተመሳሳይ ጥናቶች እነደመነሻ ግብዐት ያገለግላል። ለሀገር አቀፍ የጤና መመሪያ አውጭዎች እንደ ግብዐት በማገልገል ፤ብዙሃኑ በመረጃ ላይ የተመሰረተ እና የተሻለ ህክምና እንድያገኙም ያስችላል።

ሚስጥራዊነቱ-በዚህ ጥናት የሚሰበሰበው መረጃ በሚስጢራዊ ኮድ ሲሆን ሚስጠራዊነቱ የተጠበቀ ነው።

የመቃወምና የማቋረጥ መብት- በዚህ ጥናት ላይ የመሳተፍም ሆነ ያለ መሳተፍ ሙሉ መብትዎ የተጠበቀ ነው።

ከዚህ ጥናት ለመሳተፍ ፈቃደኛ ነዎት 1. አዎ ካሉ ይቀጥል 2. አይደለሁም ካሉ ይታለፍ

ለተጨማሪ መረጃ፡ ከጥናቱ ጋር ለተያያዘ ጥያቄ ከታች የተጠቀሱትን ሰዎች ማናገር ይቻላል፤

አለነ ጌጤነህ (ዋና ተመራማሪ) ፤ ስልክ +251-932-47-03-27 ወይም

ዶ/ር ዉዴ ምህረት (የጥናቱ ተቆጣጣሪ)፤ ስልክ +251-910-49-86-41

ከጥናቱ አቲክስ ጋር ተያዠነት ላላቸው ጥያቄዎች

የአሃሪ /አለርት ኤቲክስ ኮሚቴ ፀኀፊን ያናግሩ ስልክ + 251-113 481289

Annex VII: Case Record Form (English version)

1. Patient identification		
Patient no.______________		
Hospital admitted at		
Age ______	Sex___	Residence: Woreda_______________ kebele:________________________

2. Medical history of patient	
Types of symptoms the patient experienced?	Remark
Fever (≥38°C)	
Impaired consciousness or irritability	
Vomiting or reduced ability to suck for breastfeeding	
Stiffness of the neck or the back	
Fear of light/photophobia	

3. CSF findings	Result	Normal range	Remark
Microorganisms			
CSF protein level			
CSF glucose level			
CSF PMN %			
CSF monocyte%			
Cells count/ ul			
4. Sample appearance	❑ Turbid	❑ Clear	Other ______________

Id ………………………………………

5. Date /onset of the illness --
6. Date of admission --
7. Antibiotics usage before CSF sample collection? Yes No
8. Date of antibiotics began ---------------------------------
9. Types of antibiotics used and its durations ?(state them) --

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   ----
10. Outcomes of the patients?

a. Full recovery
b. Death
c. Unknown status

Laboratory observation

11. CSF sample collection date and time --
12. Type of culture media used to inoculate CSF

    ----
13. Inoculation date and time --
14. No growth when?

a. After 24 hrs
b. After 48 hrs
c. After 72 hrs

Annex VIII: Declaration statement

I, the undersigned Msc student declared that this thesis in titled with "Enhanced identification of GBS in infants with suspected meningitis at TASH, Addis Ababa, Ethiopia" is my original work in partial fulfillments of the requirements for the degree of masters of Science in medical microbiology. I agree to accept responsibility for the scientific ethical and technical conduct of the research project and for provision of required progress reports as per terms and conditions of the Jimma University, School of Medical Laboratory Sciences, and AHRI.

Name of the student: ____

Date.____________________ Signature _________________

This thesis work has been submitted for examination with our approval as advisors.

	Signature	date
Advisor name	----------------------	----------------
Assessors Name	---- -----------------	----------------