

Emerging of *Neisseria Meningitidis* Serogroup "C" in Gambela Region, Ethiopia

Surafel Fentaw, Rajiha Abubeker, Negga Asamene, Amete Mihret, Degefu Beyene, Elias Seyoum, Abebe Asefa, Tesfa Addis, Yonas Mekonen, Mulushewa G/Egziabher, Sintayehu Abate and Eyasu Tigabu

Ethiopian Public Health Institute, P.O.Box: 1242, Addis Ababa, Ethiopia

*Corresponding author: sura4f@gmail.com, Tel: +251-911 181729

Abstract

Background: An important bacterial meningitis causative organism, *Neisseria meningitidis*, has a high burden of morbidity and mortality in the African meningitis belt. Identifying meningococcal serotypes circulating in the region is also mandatory for appropriate vaccine introduction.

Objective: To investigate and confirm the meningitis outbreak causative agents in Gambela region, Ethiopia.

Methods: Cerebrospinal fluid samples collected from Gambela refugee camp were sent to the national microbiology laboratory (Ethiopian Public Health Institute) for etiological culture and sensitivity plus serotype identification. Specimens were cultured on Blood agar and Chocolate agar then further serotyping was done by real time PCR. Multilocus sequence typing was done at the Norwegian Institute of Public Health, Oslo Norway.

Result: Eight specimen were processed and out of these, six of them were positive for *N.meningitidis* serotype C, one Hib and one pneumococcus were identified. Genomic sequence of *N.meningitidis* serotype C showed that the strain was ST-11592, belonging to the ST-103 clonal complex, with PorA: P1.5-1, 10-4, which is similar with isolates from Arbaminch carriage study in 2014. But it was different from strain responsible for the outbreaks in Niger and Nigeria with ST- 10217 in 2015.

Conclusion: *N.meningitidis* serotype C is newly emerging outbreak causing pathogen in Ethiopia which requires vaccine together with the existing ones.

Introduction

Neisseria meningitidis is a Gram-negative diplococcus which diseases only humans. *N.meningitidis* (meningococcus) is the most important public health threat in many parts globally. The bacterium is normal flora of human beings in their nasopharynx, it sometimes gains access into the blood stream and causes systemic diseases such as meningitis, septicemia, and occasionally localized infections in the joint, and heart (Al-Tawfiq et al. 2010; Yazdankhah et al. 2014). A deadly bacterial infection once common in Africa, especially in meningitis belt of sub-Saharan Africa, stretching from Senegal in the west to Ethiopia in the east (26 countries), has the highest amounts of the disease (Yazdankhah et al. 2014). Currently, based on the immunochemistry of polysaccharide capsule, around 13 different capsular types have been identified and out of these, five serotypes are commonly isolated pathogens and the remaining can live commensally. Most meningococcal disease is caused by the five meningococcal strains of capsular polysaccharides (A, B, C, Y, and W135). At different geographical location, season, immune status of the individual and age group the distribution of the serotypes is different. The main virulence factor of the organism is potential factor by offering the pathogen inactivate the essential immunity of the alternative pathway of complement. Since the vaccine is serotype specific, vaccines are produced

against serogroups of A, B, C, W, and Y (Men A, B, C, W, and Y) and have been successfully used to control these serogroups of meningococci. Capsulated or unencapsulated strains are the natural reservoir of the human nasopharynx (Harrison et al. 2011; Shao et al. 2006) In addition to the capsule, serotyping of *N. meningitidis* could be done based on the variable regions of the genes (porA, porB, and fetA) encoding the outer membrane proteins. But the surface antigens and their genes could be influenced by selective pressure and that makes them greater diversity which made them suitable only for short term or localized epidemiological tracking. In contrast, housekeeping enzymes and their genes are neutral (not under selection) and are therefore, perfect markers for long term and global epidemiology. Multilocus sequence typing (MLST) used to characterize meningococcal isolates using the non-cell surface components such as housekeeping genes (abcZ, adk, fumC, gdh, pdhC, and pgm). Identifying meningococcal serotypes circulating in the region is also mandatory for appropriate vaccine introduction as well as molecular epidemiology of pathogens (Findlow et al. 2007). The aim of this paper focuses identifying meningococcal serotypes that are emerging and become public health importance in Ethiopia.

Materials and methods

Study population and bacteriological samples: In 2015, the dry season between February and April there was meningococcal outbreak, in Gambela region, Gambela, Ethiopia. In order to identify and confirm the meningitis outbreak causative agents, laboratory investigation was mandatory. For this Cerebrospinal fluid (CSF) samples were collected from Gambella Region refugee camps. Samples were transported to Ethiopian Public Health Institute (EPHI) using Trans Isolate transport media at ambient temperature. All CSF specimens in Trans isolate media were analyzed by culturing for the presence of *N.meningitidis* immediately upon arrival at EPHI. The specimens were inoculated on blood

agar plates (Oxoid, Bangshire, England) containing 5% sheep blood, and on chocolate agar plates (Oxoid, Bangshire, England). The plates were incubated at 35°C under 5-8% CO₂. Suspected colonies were identified as meningococcus, pneumococcus or hemophilus by Gram stain followed by oxidase, catalase (3% H₂O₂) and optochin test. According to the preliminary findings complementary test was done by latex agglutination test (pastorexmeningitis^R). Finally confirmatory test was done by Real-Time PCR using ctr A, hpd and lytA primers for meningococcus, hemophilus and pneumococcus respectively (Table 1).

Table 1: Real Time primers and probes used for detection of bacterial meningitis

Target	Primer or Probe name	Real-time Primers and Probes Nucleotide Sequence (5' to 3')	Probe Modifications
<i>N.meningitidis</i>			
ctrA	ctrA-F ctrA-R ctrA-P	GCTGCGGTAGGTGGTTCAA TTGTGCGGGATTGCAACTA CATTGCCACGTGTCAGCTGCACAT	5'-FAM, BHQ1-3
<i>H. influenza</i>			
Hpd	hpdF822 hpdR952 Pb896i	GGTTAAATATGCCGATGGTGTG TGCATCTTTACGCACGGTGTA TTGTGTACACTCCGTXGGTAAAAGAACTTGCAC	5' FAM, BHQ1-dT on X, 3' SpC3
<i>S.pneumoniae</i>			
lytA	F373 R424 Pb400i	ACGCAATCTAGCAGATGAAGCA TCGTGCGTTTTTAATCCAGCT TGCCGAAAACGCXTGATACAGGGAG	5' FAM, BHQ1-dT on X, 3' SpC3
Thermal cycling condition		95oC for 20 Sec 95°C for 3 se 60°C for 30 sec	1cycle 45 cycles

Serotyping: Isolated meningococcus species were serotyped at the EPHI by Real-Time PCR using the following gene specific primers probes (Table 2).

Table 2: Real-time Primers and Probes (singleplex RT-PCR reaction)

Target	Primer or Probes	Neucleoside sequences (5 to 3')	Probe Modifications
NmA	F2531 R2624 Pb2591i	AAAATTCAATGGGTATATCACGAAGA ATATGGTGAAGCTGGTTTCAATAG CTAAAAGXAGGAAGGGCACTTTGTGGCATAAT	5'-FAM, BHQ1-dTon X, SPC3-3
NmB	F737 R882 Pb839i	GCTACCCATTTCAGATGATTGT ACCAGCCGAGGGTTTATTCTAC AAGAGATGGGYAACAACXATGTAATGTCTTTATTT	5'-FAM, BHQ1-dTon X, SPC3-3
NmC	F478 R551 Pb495i	CCCTGAGTATGCGAAAAAATT-3 TGCTAATCCCGCTGAATG TTTCAATGCXAATGAATACACCGTTTTTTTGC	5'-FAM, BHQ1-dTon X, SPC3-3
NmY	F787 R929 Pb1099i	TCCGAGCAGGAAATTATGAGAATAC TTGCTAAAATCATTGCTCCATAT TATGGTGXACGATATCCCTATCCTTGCCCTATAAT	5'-FAM, BHQ1-dTon X, SPC3-3
NmW	F857 R964 Pb907i	TATTTATGGAAGGCATGGTGTATG TTGCCATTCCAGAAATATCACC AAATATGGAGCGAAXGATTACAGTAACTATAATGAA	5'-FAM, BHQ1-dTon X, SPC3-3
NmX	F173 R237 Pb196	TGTCCCAACCGTTTATTGG TGCTGCTATCATAGCCGCC TGTTTGCCACATGAATGGCGG	5'-FAM, -BHQ1-3

Antimicrobial susceptibility testing: For all confirmed meningitis etiologies, antimicrobial susceptibility was performed by Minimum inhibitory concentration (MIC) using Etest MIC (Biomeriux, France). The Clinical and Laboratory Standard Institute (CLSI) guideline was used for interpretation.

Genotyping: Genomic DNA from each bacterial isolate was obtained after boiling a 1-ul loopful of colony material grown over night on Blood agar after suspending this in 100µl Tris EDTA buffer for 10 minutes in phosphate buffer saline at 100°C in a dry bath followed by centrifugation at 13,000 rpm for 5 minutes. The supernatant was removed and stored at -80°C until shipped to Norwegian Institute of Public Health (NIPH) used for Multi Locus Sequence Typing (MLST). MLST was performed using the target

housekeeping genes (*abcZ*, *adk*, *aroE*, *fumC*, *gdh*, *pdhC*, and *pgm*) (10). Then Sequence Types (ST) were assigned according to the combination of the seven alleles with

reference to the *N. meningitidis* MLST database (www.mlst.net). Classification by outer membrane protein was done by sequencing of the *porA* and *fetA* genes.

Results

All participants were from Gambela Refugee camps and out of these 5 male and 3 female with the age of 23-32 years. All specimens were culture positive for meningococcus, pneumococcus and Hib with a frequency of seven, one and one respectively. Meningococcal isolates were positive for serogroup C meningococcus (Table 3).

Table 3. Microbiology culture results and serotype distribution of *N. meningitidis* in Gambella, Ethiopia, 2015.

Test	Frequency
Culture	
Positive	8
Negative	0
Real Time PCR	
Meningococcus (<i>ctrA</i>)	6
Pneumococcus (<i>lytA</i>)	1
Hib (<i>hpd</i>)	1
Meningococcal serotype	
Serogroup A(<i>mynB</i> / <i>sacB</i> (A))	7
Serogroup C(<i>siaD</i> (C))	0
Serogroup W135 (<i>siaD</i> (W135))	0
Sequence type	11592
<i>PorA</i>	P1.5-1,10.4

Meningitis causing agents were tested for available antimicrobial agents and none of the isolates were resistant for tested antimicrobial agents (Table 4).

Table 4: Antimicrobial Susceptibility pattern of meningitis etiologies in Gambela, Ethiopia, 2015.

Organism	Antibiotics Minimum Inhibitory Concentration		
	Penicillin	Ceftriaxone	Chloramphenicol
Meningococcus	0.008±0.02	0.041±0.02	0.5 ±0.02
Pneumococcus	0.008±0.02	0.12	2
Hib	-	0.12	1

Discussion

In Ethiopia, meningococcal polysaccharide vaccines A has been used for routine or mass immunization. In many African countries, repeated vaccination against *N. meningitidis* serogroups A has likely led to a selective increase in the incidence of meningococci of other serogroups. This will subsequent in a transformed profile of meningococcal disease. In recent years, invasive disease caused by *N. meningitidis* serogroup W135 and serogroup C strains have emerged in the African meningitis belt. (Warshawsky et al. 2013, Bärnes et al. 2016). Therefore, meningococcal disease caused by

serogroups other than *N. meningitidis* A strains appears to be an emerging problem and should be investigated epidemiologically.

Antibiotics are given to individuals who have complicated meningitis or individuals of close contact to patients with meningitis. All identified meningitis agents identified during the Gambela outbreak were susceptible for tested antimicrobial agents.

Genomic sequence of *N. meningitidis* serotype C found in Ethiopia during 2015 meningitis outbreak in Gambela region showed that the strain was sequence type of ST-11592, belonging to the ST-103 clonal complex, with *PorA*: P1.5-1, 10-4. The genomic characteristics of this strain was similar with *N. meningitidis* serogroup C isolates from ArbaMinch Ethiopia (Southern Ethiopia) carriage study in 2014 (Bärnes et al. 2016) But the sequence type of *N. meningitidis* serogroup C in Gambela, Ethiopia, was different from *N. meningitidis* serogroup C strain responsible for the outbreaks in Niger and Nigeria ST- 10217 in 2015 (Cecilia et al. 2016).

Conclusions and Recommendation

The outbreak in Gambela was caused by *N. meningitidis* serotype C. It is the newly emerging burden in Ethiopia which needs the focus of policy makers. Based on this finding, Trivalent conjugate meningococcal vaccine was introduced to Gambela for intervention. For the future, enhanced meningococcal surveillance should be in place in order to identify the existing serotypes circulating in the community.

References

- Al-Tawfiq JA, Clark TA & Memish ZA (2010). Meningococcal disease: the organism, clinical presentation, and worldwide epidemiology. *Journal of Tropical Medicine*, 17(sup – 1): 3–8.
- Bärnes GK, Kristiansen PA, Beyene D, Workalemahu B, Fissiha P, Merdekios B, et al. (2016). Prevalence and epidemiology of meningococcal carriage in Southern Ethiopia prior to implementation of MenAfriVac, a conjugate vaccine. *Bio Medical Centre Infectious Diseases*, 16:639.
- Cecilia BK, Retchless AC, Sidikou F, Issaka B, Ousmane S, Schwartz S, et al. (2015). Whole-Genome Characterization of Epidemic *Neisseria meningitidis* Serogroup C and Resurgence of

- Serogroup W, Niger. *Emerging Infectious Diseases*, 22(10): 1762-1768.
- Findlow H, Vogel U, Mueller JE, Curry A, Njanpop-Lafourcade BM & Claus H (2007). Three cases of invasive meningococcal disease caused by a capsule null locus strain circulating among healthy carriers in Burkina Faso. *Journal of Infectious Disease*, 195(7):1071–1077.
- Harrison OB, Brueggemann AB, Caugant DA, van der Ende A, Frosch M, Tray S, et al. (2011). Molecular typing methods for outbreak detection and surveillance of invasive disease caused by *N. meningitidis*, *H. influenzae*, and *S. pneumoniae*, a review. *Microbiology*, 157(Pt 8): 2181–2195.
- Shao Z, Li W, Ren J, Liang XF, Xu L & Diao BW (2006). Identification of a new *Neisseria meningitidis* serogroup C clone from Anhui Province, China. *Lancet*, 367(9508): 419–423.
- Warshawsky B (2013). Update on the use of quadrivalent meningococcal vaccines. An Advisory Committee Statement (ACS) National Advisory Committee on Immunization. *Canada Communicable Disease Report Monthly*, 39 (1).
- Yazdankhah SP, Kriz P, Tzanakaki G, Kremastinou J, Kalmusova J, Musilek M, et al. (2014). Distribution of serogroups and genotypes among disease-associated and carried isolates of *Neisseria meningitidis* from the Czech Republic, Greece, and Norway. *Journal of Clinical Microbiology*, 42(11): 5146–5153.

