Meeting of WHO Collaborating Centres for Meningitis

WHO Headquarters, Geneva, September 2017

The WHO meningitis team outlined the epidemic meningitis control strategy, highlighting the importance of strengthening meningococcal disease surveillance in African Meningitis belt countries. This remains a priority area requiring continued support and collaboration from the WHO Collaborating Centres (WHOCCs). Particular key focus areas for continued collaboration are: building meningitis diagnostic capacity, strengthening molecular surveillance, and ensuring the evaluation of the laboratory performance of African meningitis belt laboratories.

Each of the three institutions then summarized the activities they conducted in their role as WHOCC and also presented one activity in depth.

The Centers for Disease Control and Prevention **(CDC), Atlanta** summarized their activities, with a focus on supporting laboratory diagnostic capacity building in several belt countries. They continue to provide support for polymerase chain reaction (PCR) testing of meningitis specimens, including trainings, technical assistance and procurement of laboratory materials and equipment. They have developed a local procurement mechanism for PCR supplies in Burkina Faso, Niger and Chad, fostering sustainable, in-country procurement solutions. CDC also provided technical assistance during outbreak investigation and response in Nigeria, Ghana and Liberia.

CDC highlighted their work in the outbreak investigation of a cluster of unexplained illness in Liberia, in May 2017. Thirty one cases were reported, including 13 deaths. Ebola virus disease and Lassa fever were initially suspected. Hematology and chemistry tests on specimens from the cases did not reveal any significant abnormalities, and all specimens tested for Ebola and Lassa virus by PCR were negative. Using Taqman Array Card (TAC), which allows simultaneous detection of multiple pathogens, and direct real-time PCR, CDC very rapidly identified *Neisseria meningitidis* (Nm) C as the causing pathogen. Metagenomic analysis determined that 6 of 10 specimens from six cases had >90% similarity to the Nm C ST-10217 strain that emerged in 2013 and caused large epidemics in Niger and Nigeria. Given the unusual characteristics of these cases (high attack and case-fatality rate and predominance of gastrointestinal symptoms), an epidemiological description of this meningococcal outbreak should be disseminated for future outbreak detection and management. This experience in an African country outside the meningitis belt, raises the question of the level of surveillance and preparedness that should be recommended for 'lower risk countries'.

The National Institute of Public Health (**NIPH), Oslo** has been providing support in laboratory confirmation and meningococcal strain characterization of samples from countries of the African meningitis belt. From 2015 to 2017 they received 510 samples from Democratic Republic of Congo, Ethiopia, Nigeria and South Sudan. Of these, 20% were positive for one of the three main bacterial

meningitis pathogens by either culture or PCR. Whole genome sequencing analysis of Nm C strains from Niger and Nigeria (2013 to 2017), indicates that their evolution is not linear. The Nm C strain has spread in Nigeria, probably through carriage, acquiring new variation and resulting in the constitution of 2 lineages or clades. NIPH provided technical support to reinforce laboratory diagnosis in Ethiopia, including training, elaboration of standard operating procedures, procurement of real-time PCR (rt-PCR) equipment and supplies. While the confirmation rate in the Ethiopian laboratories was low (4.5% positive samples compared to 18.5 % in NIPH lab), a special study in Jimma, using FilmArray assay allowed the identification of many other pathogens including several viruses. A meningococcal longitudinal carriage study they conducted, also in Ethiopia, found frequent genetic changes in the meningococcus short-term carriage, most often due to phase variation.

NIPH provided diagnostic support for a carriage study in Niger, using multiplex rt-PCR to detect Nm using three targets: *porA, sodC* and *cnl*. This method did not perform well in detecting *Nm* serogroups, with low sensitivity of *porA* rt-PCR and low specificity of *sodC* and *cnl*. As throat swab samples are not sterile, as cerebro-spinal (CSF) fluid is, many commensals including *Neisseria lactamica* are found. This study suggests that culture may still be a better method than rt-PCR when conducting carriage studies.

The Institut Pasteur Paris (IPP) transferred bacterial meningitis diagnostic tools and technologies through training in CAR, Cameroon, Côte d'Ivoire, Niger and Morocco. They also contributed to outbreak investigations in Uganda, Cameroon and Liberia. The outbreak in Uganda was atypical for the region, with a majority of cases of acute bacterial conjunctivitis, several cases of acute bacterial meningitis, as well as some pneumonia cases. Antibiotic susceptibility testing performed on the conjunctivitis isolates in Uganda, found a concerning reduction in susceptibility to penicillin and other beta lactams. However, molecular sequencing performed by IPP (as no cultured isolates were sent) indicated that meningococcal isolates were likely fully susceptible to beta lactams. During the Cameroun outbreak in Yaoundé prison, IPP conducted culture and PCR tests confirming 6 Nm C and 11 Nm W samples. The Nm C strain was found to be of ST-2881, belonging to clonal complex 175 and often found in carriage, in South Africa, and frequently in serogroup W. This is a less invasive sequence type than the ST-10217 from Niger and Nigeria. Plasma samples from the Liberia outbreak were tested by ELISA serological analysis and a significant immune response to Nm C was found - pointing to probable recent Nm C infection. The confirmation of meningococcal disease with unusual characteristics for the African region highlights the importance of including meningococcal septicemic presentations in the surveillance case definitions in Africa.

IPP conducted a study to evaluate the performance of the PneumoSpeed lateral transfer cassettes in detecting *Streptococcus pneumoniae,* under laboratory conditions. The sensitivity and specificity of PneumoSpeed using 18 CSF samples were found to be 94.4% and 100%, respectively. It took <1 minute to obtain the results. Urine from mice was also tested using PneumoSpeed with good results, suggesting that field-testing using urine samples should be pursued.

In 2017, the **WHO Invasive Bacterial Vaccine Preventable Diseases program for External Quality Assurance** included 15 laboratories from countries of the African meningitis belt which are supported by the WHO Collaborating Centers for meningitis. Full panels (which include both culture and PCR

specimens) were sent to the large majority of the laboratories except for two laboratories which were already enrolled and had been receiving partial panels (including culture but no PCR). Only 8 of the 15 laboratories completed the exercise and submitted results. Individual reports are being elaborated and will be shared with each laboratory. Once these reports are distributed, the respective WHO CCs, in coordination with the WHO meningitis team, will review the results with the respective laboratories and propose corrective actions when needed.

Plans for a joint WHO CC publication on molecular surveillance results from 2011 to 2016 isolates were discussed. The three WHOCCs will share their individual lists of 2011-2016 meningococcal strains in a previously established common platform and NIPH will compile them into a single database. Each WHOCC will sequence their respective isolates and the raw sequence data will be transferred to CDC for analysis. The objective of the analysis will be to monitor the evolution of invasive meningococcal disease strains (particularly Nm C and Nm W) through whole genome sequencing.