Global Task Force on Cholera Control (GTFCC) Working Group on Laboratory Surveillance

Webinar 01, 22 January 2021

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Acronyms and abbreviations

GTFCC	Global Task Force on Cholera Control
MIC	Minimum Inhibitory Concentration
NCP	national cholera control plan
OCV	oral cholera vaccine
PCR	polymerase chain reaction test
SOP	standard operating procedure
WASH	water, sanitation and hygiene
WHO	World Health Organization

Participants

Amanda Debes
Anthony Okon
Anthony Smith
Asish Mukhopadhyay
Christopher Friedrich
Ciglenecki Iza
David Olson
David Sack
Francisco Luquero
Ghassan M. Matar, PhD
Jibir Zanguina
Marianne GOJON-GERBELOT
Marie-Laure QUILICI
Mark Nunn
Maryann Turnsek
Michele Parsons
MUNIRAL ALAM
Myriam Henkens
Nick Thomson
Valentina Picot
Wandani Sebonego

Introduction

***David Olson**, GTFCC Secretariat cholera team*

Dr Olson welcomed several new members to the group.

Brief overview of 2019-20 output

***Marie-Laure Quilici**, Working Group Chair*

Job aids have been completed for domestic and international transportation of cholera samples and strains. Guidelines for transporting domestic samples for laboratory confirmation of *Vibrio cholerae* have been edited in English and French and included in WHO cholera investigation kits available on GTFCC application. The guidelines for international transportation, also now available in English and French and included in the WHO cholera lab kit, describe the conditions of strain conditioning and shipment internationally, including sample preservation, preparation before sending, and the logistical and administrative aspects of shipment.

A further job aid has been completed in English for antimicrobial susceptibility testing for treatment and control of cholera. The French version was in editing at the time of the meeting, with an associated fact sheet and possibly standard operating procedures (SOPs) to come.

A job aid sheet on *V. cholerae* culture procedure was in the process of being formatted at the time of the meeting, for later review by the working group. A further culture fact sheet is to follow.

The WHO cholera lab kit has been updated in order to bring it in line with the new job aids.

The development of specific guidelines for antimicrobial resistance to *V. cholerae* raises a number of questions that have not yet been settled, with two differing references for interpretation (CLSI and EUCAST) to be resolved.

RDT performance review

Amanda Debes

Dr Debes presented a draft of a performance review of rapid diagnostic tests (RDTs) for cholera, which she described as in its "early days" and still in need of a "collective process to reach baselines on these items." The objectives of the project were a literature review of RDT performance; research on the role of enrichment; examination of the use of RDT in different prevalence contexts; and the development of a standardized field RDT performance evaluation protocol.

Variables of interest included RDT type; the country or region in which it was tested; whether evaluation had taken place in the laboratory or in the field (and if the latter, where testing was conducted); the training of the person performing the test; the situation in which the RDT was being used (i.e. in the beginning, middle or end phases of outbreak, or for surveillance); whether the direct or enriched method was used; the type of confirmatory test; the use, or not, of antibiotics; and "faint line considerations" around the subjectivity of interpreting the tests themselves.

Across the literature, RDT sensitivity outperformed acceptable minimums, but specificity fell short, "a true demonstration of how we are seeing specificity in direct applications in the field." Not all studies included enrichment, but it was evident across studies that the high sensitivity + high specificity combination was unusual. Potential explanations for wide variations in sensitivity and specificity include patient exposure to antibiotics or phages. In discussion it was mentioned that a practical response to this finding in field applications would be to de-emphasise a negative RDT result in cases where the patient is known to have taken antibiotics and focus on clinical presentations. Phages were described as the "great unknown" – exploratory work is under way on an RDT with second line for monoclonal antibodies for most common vibriophage, with the hope of a diagnostic study starting next year.

As an explanation of the discrepancies between the culture and RDTs results, it was also pointed out that when samples are plated twice during laboratory diagnosis of cholera, it is not uncommon for one plate to return a positive result and the other negative, due to the low load of cholera Vibrios in the sample which can explain a negative RDT result on an ultimately cholera positive sample. The differences in results between enriched and direct methods should also not be surprising: when doing culture, enrichment can be positive and direct negative, but the opposite has also been observed.

There was discussion of thresholds for probability required to use clinical case definitions combined with RDT results enough as the basis for declaring outbreaks. Some of the answer hinges on the likelihood of RDTs being available: if they are included in WHO kits, then this could be a good approach; but in reality RDTs may not be readily available in real situations. Situation-specific guidelines may therefore be required.

Dr Debes presented a review of the different situations and criteria applicable when considering the use of RDTs at different stages in an outbreak (at initial detection and confirmation of an outbreak, at the end of an outbreak, for outbreak monitoring, and for surveillance of burden of disease), a table of the probability of at least one true cholera positive among 10 patients tested via RDT by prevalence, and a table of the probability of at least one true cholera case per given number of patients tested via RDT. Risk thresholds are a central consideration in practical terms: in low prevalence situations it is possible to declare an outbreak with a small number of samples if the given risk of falsely declaring an outbreak is considered to be acceptable. If the probabilities are known, it is possible to estimate the risk of a false declaration from a given proportion of positive tests, and based on different thresholds of acceptable risk, different recommendations can be made.

There was discussion of a proposed standard performance evaluation protocol, and the role of RDTs in determining the likelihood of biologic presence of transmissible toxigenic *V. cholerae* O1/O139. Interpretation is influenced by test characteristics and context, and there is certainly the possibility or more and better data on probabilities and prevalences. Settling on a field evaluation protocol has been challenging, with a number of questions around situation specific protocols; defining sample sizes to get specific levels of precision/power in results; whether or not to recommend enrichment (while there are strong arguments for doing this, it is an extra step and in reality it is often not done even when recommended); settling on recommended confirmation options; and whether the study should use different people for field application of RDTs and laboratory confirmation (realistically there is some subjectivity in interpreting RDTs). If a protocol was published on how performance evaluations should be conducted, it would involve more than just following the instructions in manufacturer inserts. Who will be supporting this effort is also important – whether it is backed by countries, collaborators, manufacturers or some combination, this will affect the potential asks around funding and other issues.

2021 GTFCC Lab Surveillance Working Group Work Plan

***ML Quilici**, Working Group Chair*

***David Olson**, GTFCC Secretariat cholera team*

The priorities of the proposed 2021 work plan are organised around four themes.

Minimum country lab standards/capacity

- Define the minimum essential technical capacity for countries actively engaged in cholera prevention and control
- Roll out Questionnaire to survey NCP-engaged countries on needs for minimum technical capacity

For minimum lab capacity standards, it is proposed that each country should have at least one central lab able to identify toxigenic V cholerae O1 (by culture and/or PCR) and perform antibiotic resistance testing to nalidixic acid/ciprofloxacin, tetracycline and azithromycin. RDTs should be available in every high-risk hotspot district, stored at district level or in health facilities, for detection and monitoring of new and ongoing outbreaks and to achieve standardized acute watery diarrhoea (AWD) surveillance over time. Laboratory data should be integrated (by date and district location) into national surveillance systems through systematic reporting of numbers of suspected cases tested, positive and negative results and the method used in testing (RDT, culture and/or molecular). There should be no cumulative stockouts or key personnel missing for longer than two weeks in a year, and quality assurance programmes and annual refresher training and evaluation, including for RDT use, should be in place. Genomics testing and analysis training is not essential at first but even if not included in the minimum country lab standard, measures should be considered to achieve it (such as agreements or collaborations with partners able to perform these analyses).

Antibiotic sensitivity testing

- Produce SOPs for antibiotic sensitivity testing in support of the AMR (AMS) Job-aid
- Monitoring of antibiotic sensitivity patterns over time and place, and how to share them in real time because of the immediate impact on the treatment of cases and the management of epidemics
- Adding antimicrobial resistance (AMR) data to long-term cholera database

As mentioned earlier, the AMR (AMS?) job aid that has been prepared will be circulated to the working group for final approval. How to access interpretation criteria of the two international standards (CLSI vs EUCAST) is still to be decided (link to the website versus table of values, knowing that the CLSI data is subject to copyright). A table of values could be presented in the SOPs but not on the job-aid, which would also make it easier to modify in the future if needed.

PCR testing

- Defining a standard protocol (real-time vs conventional)
- Making judgements around use of commercial tests vs in-house approaches

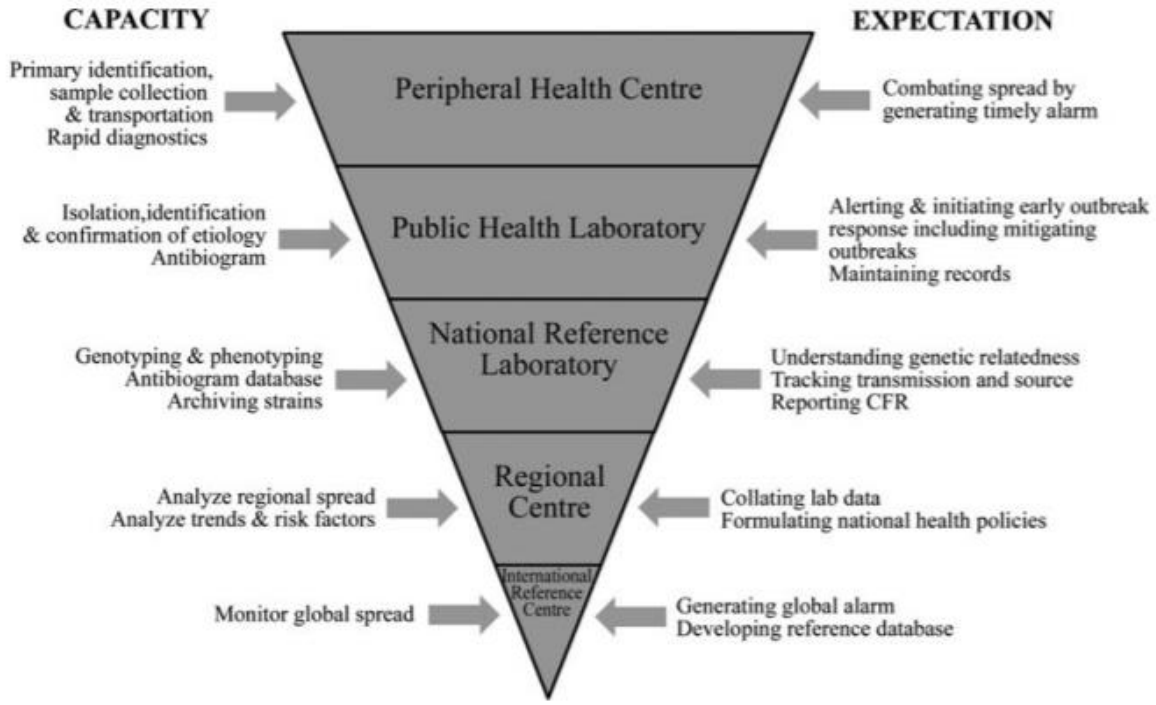
It is necessary to develop capacities for PCR testing in all countries. There is no standard protocol, and commercially available kits for real time PCR are expensive (at approximately 90€ per test) and require the use of specific and limited models of thermocyclers. A shared working group folder has been created to collect PCR procedural manuals already used by laboratory partners. These include those used by the US Centers for Disease Control and Prevention CDC (conventional PCR); Institut Pasteur (conventional); the Johns Hopkins School of Public Health (conventional); the South Africa National Institute for Communicable Diseases (real time PCR); and the Indian National Institute for Cholera and Enteric Diseases (conventional). David Olson will provide the necessary information for the deposit of the SOPs of other partners in the dropbox created.

Defining the role of the working group in the epidemiology working group sub-groups.

It will be necessary to determine how laboratory capacities can be used to improve identification of true preponderance of cholera during and in between outbreaks, closing existing gaps between lab and epidemiology work. The role of lab testing in regional/global cholera spread should be defined, as should protocols for environmental testing for toxigenic *V. cholerae* detection and the role of labs in determining and validating cholera elimination. The GTFCC surveillance guidelines also require revision.

Additional priorities

Dr Olson also presented a slide on the development of a Grand Diagnostic Strategy (see below) – a worthy goal for 2021, which could be based on the following proposed scheme:



Discussions

All of these priorities should be decided according to country requests, so information is requested on countries' gaps and needs.

The questionnaire on lab capacity is planned not with regard to materials, but rather to technical possibilities, in term of personnel and capacity (training). The group was invited to respond to the proposed sets of standards presented, and the representatives of each country concerned to subsequently offer information on countries' current positions when compared to these standards.

In discussion, it was agreed as part of the minimum standard that the lab data integration aspect should be included. It was pointed out that the idea of specimen transportation should be included also: too often specimens are collected in hotspot areas with limited systems to get them to a place where they can be subjected to additional higher level testing. It might therefore be worth addressing possibilities for integrating with existing specimen referral capacities that might be in place with other networks – for example using postal systems, motorcycles, HIV/TB networks already moving samples, etc. rather than developing new, vertical systems.

Further work with manufacturers is needed to get RDT products to meet required performance specifications. Prequalification processes may prove useful in this regard.

There are still many further discussions to be had on the issue of antibiotic resistance and AMR monitoring. A job aid proposed does not answer all the possible questions. Countries have requested more in this area, and there is a need to discuss interpretation criteria further – possibly in a separate webinar. Definitions of interpretation criteria that are coherent among the two

international standards (CLSI vs EUCAST) are still to be decided, and the choice between maintaining the disc diffusion methodology vs MIC determination (test strips or microplates), which is increasingly recommended, could also be to discuss .