**Additional Questions and Answers**

**What types of bioinformatics tools are recommended for virulence factor and AMR gene identification for metagenomics and metatranscriptomics data?**

There are various bioinformatics tools and approaches depending on the sequencing platform and the specific research questions. For species-agnostic profiling across entire datasets, platforms such as CGE Metagenomics and EPI2ME (for nanopore sequences), or CZ-ID (for both Illumina and Nanopore reads) are recommended. If the objective is to link AMR/Virulence genes to specific microorganisms, a more targeted workflow involving metagenome assembly and taxonomic binning to generate MAGs, and subsequent screening of MAG fasta files with tools like AbritAMR or Abricate against databases such as NCBI AMRFinder and VFDB is recommended.

**How are hospitals balancing the use of last-resort antibiotics like colistin with the risk of toxicity to patients?**

If a patient has a bacterial infection that is resistant to all antibiotics then colistin is a last-resort drug that is very effective. Colistin can cause acute kidney injury and neurological issues so careful consideration of the patients overall health is critical and a cost benefit analysis must be undertaken. Side effects can be managed but death is irreversible. The patient is carefully monitored when the drug is delivered and the dosing is titrated to delvier the lowest effective dose. Colistin prescription should be regulated through the AMS teams within the hospital to ensure that all antibiotic options have been exhausted.

**Having many vaccines at hand and the majority being imported, should we not be assessing the effectiveness, since there are many environmental factors that differ between Europe and Africa? How often should the effectiveness of the vaccines be measured?**

Yes, vaccine developers should utilize strain/serotype data from the countries where the vaccines will be delivered to ensure that they are effective against the endemic strains. Once the vaccines are deployed, there should be frequent monitoring to ensure that the endemic strain composition has not changed. Ideally, monitoring should be continuous, but an annual cross-sectional survey should give a good indication of the continued effectiveness of vaccines. A good example is the flu vaccine, which is changed yearly based on the dominant strains in the prior flu season in different hemispheres. COVID taught us that factors such as cold-change availability and stability impact the effectiveness of the vaccines, and we know that exposure to different diseases can skew our immune systems and impact how effectively we can mount immunity against other infections. These are some additional factors that should be accounted for. Vaccine clinical trials are tested in the countries where they will be deployed to account for these additional factors.

**How do we contain AMR in a poor and dilapidated environment, with high levels of spread of salmonella or any associated infection many people are left untreated due to high levels of infection, contamination, and poverty.**

Antibiotics, unfortunately, have been used as a replacement for basic practices that are effective in limiting the spread of bacterial infections. In the absence of these basic structures and practices it's losing battle. Some of these are:

- Availability of clean running water in hospitals and communities
- Hand washing
- Frequent cleaning of surfaces and especially high touch areas with soap and water or disinfectant
- Good national health systems that ensure access to Level 1-3 antibiotics for its citizenry
- Good farming and food processing practices
- Appropriate waste management systems

**You indicated that we should be concerned with plasmids and their ability to enhance bacterial ability to develop multiple genes that confer resistance to various classes of antibiotics. Does bacterial activation before sequencing have an impact on the presence of plasmids? Do bacteria lose some in the process? And what would be the repercussions, especially on AMR genomic surveillance.**

It is unclear what you mean by "bacterial activation". If one extracts the total genomic DNA from a bacterium and then does whole genome sequencing, then both the plasmids and chromosomal DNA will be sequenced. However, if one does short-read WGS on Illumina, it may be difficult to tell if the AMR genes are located on the plasmid or chromosome because of the loss of genomic contextual information. One of the strengths of the Oxford Nanopore long reads is that you can sequence an entire plasmid in a single strand, so it is clear which AMR genes are on the plasmid.

If the location of ARGs are not clear the risk of resistance spread cannot be determined which has an impact on the interpretation of surveillance data. As we discussed ARGs on plasmid can move very fast within and between species so represent a greater risk of increased AMR

Plasmids can be lost when bacteria are subcultured repeatedly in the lab. Good practice is to sequence low-passage bacteria for isolation from patient samples.

If plasmid sequences are missed in the sequencing approach, then we lose the largest contributor of ARGs, and this can lead to erroneous genomic surveillance results and erroneous discordance between phenotypic and genotypic data.