Antimicrobial Resistance and Whole Genome Sequencing –

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Need of WGS in AMR survalliance

- The fast spread of antimicrobial resistance (AMR) and lack of alternative antibiotics have been declared as a global public health emergency
- The greatest impact of AMR could be seen in poor - setting, where the prevalence of multidrug-resistant pathogens is growing
- whole-genome sequencing (WGS) as a new tool has been shown as a very potential approach that is applicable for many kind of genomic investigations
- In term of AMR, using whole-genome sequencing (WGS) for molecular surveillance can be a valuable addition to phenotypic surveillance of AMR.

WGS provides insights

- into the genetic basis of resistance mechanisms,
- pathogen evolution and
- Population dynamics at different spatial and temporal scales.
- (NIHR Global Health Research Unit on Genomic Surveillance of AMR. Wholegenome sequencing as part of national and international surveillance programmes for antimicrobial resistance: a roadmap. BMJ Global Health 2020;5:e002244. doi:10.1136/ bmjgh-2019-002244 Handling editor Seye Abimb)

Need of WGS in AMR survalliance

- The high cost and complexity of performing of WGS is reason why mainly it is carried out in high-income countries.
- the potential of this approach to inform national and international action plans against AMR, makes establishment of WGS as a surveillance tool very important both for our country and internationally

Main tasks that should be consider are

- defining a roadmap to how to establish and implement it as complementary to phenotypic testing
- overcome obstacles to the implementation it in Low and Middle income countries(technical support, financing)
- Need of collaborations between high-income countries and LMICs at various stages of implementing WGS for AMR surveillance

WGS advantages

- huge improvement in understanding mechanisms of how bacteria can become resistant to antibiotics.
- **Prediction of** AMR more efficiently using a single WGS workflow.
- **confirmed a high correlation between the presence of AMR genes and the phenotypic data.**
- Additionally some new tools such as metagenomics are focusing on applying sequencing to the sample itself, eliminating the need to isolate and sequence bacteria from the sample.

The genomic sequencing technology could be directly apply to a test sample and the advan

- tages are**:**
- **reduction of an analytic time**,
- **increasing the efficiency of the intended outcome** (surveillance or therapy)
- **Significant lowering the cost savings.**

WGS advantages

- Taking all together it could be expected that WGS aproach for AMR survalliance replace the phenotypic testing for some aspects of their surveillance programs and in many countries will begin to rely only on WGS analysis
- Many results obtained from WGS of bacteria are already analyzed for the presence of AMR genes and uploaded into a public database hosted by the National Center for Biotechnology Information (NCBI) at NIH.
- Therefore, the data are public available and can access this information including the type of AMR genes present.
- WGS coupled with the *One Health* may allow deeper level of understanding the AMR and thus increase the longevity and even efficacy of currently used antibiotics

- In conclusion WGS data gained for from a single test yields far more information than other methods (PCR or microarrays) and, at its most fundamental level, and does not require previous knowledge of the resistance phenotype of the isolate.

Cautions

- WGS it is no simple task, especially when the data have been generated by short-read ('second generation') technology
- What we must note is that sequencing data fromWGS could be produced from different sequencing platforms (Illumin, NanoporeOxford). There are difference between short and long read approach in terms of accuracy
- Short read (100 to 500 bp) output with high accuracy may be complemented by that produced by much longer reads.
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- The short-read technology produces single -raw reads that are in most cases shorter than the genes responsible for the reduced susceptibility to a given antimicrobial agent and either need to undergo *de novo* assembly to obtain larger fragments of the originally contiguous DNA -'contigs' **or by reference ('mapping') to known** genetic targets
- Repetitive regions of bacterial genomes are particularly challenging and there and correct assembly might be problematic.
- Only data sets that pass agreed QC metrics should be used in AST predictions. Minimum performance standards should exist and comparative accuracies across different WGS laboratories and processes should be measured.

Need for databases and limitation of performance

- To facilitate comparisons, a single public database of all known resistance loci should be established, regularly updated and strictly curated using minimum standards for the inclusion of resistance loci.

- major limitations to widespread adoption for WGS-based AST in clinical laboratories remain the **current high-cost and limitedspeed of inferring antimicrobial** susceptibility from WGS data
- as well as the dependency on previous culture because analysis directly on specimens remains challenging
- Additionalythere is a need to understand the potential 'added value' of WGS **regarding the clinical implications of AMR and**
- so the validity of data generated by WGS **must be challenged against phenotypic methods**to differentiate S isolates from R isolates.

Why to implement WGS as surveillance with examples

- Patients taking antiretroviral treatment against HIV infections, need to do it regularly
- Otherwise, the virus can become resistant. As a consequence, patients have to change therapy to different drugs, which may be difficult to obtain, more expensive, or might have more side effects.
- Lack of action to prevent, monitor and respond to HIV drug resistance can block the world from meeting the main targets, to ensure that 95% of people receiving HIV treatment achieve suppression of the virus in their blood by 2030.
- This approach enable to prevent, monitor and respond to HIV drug resistance and to accelerate progress towards achieving the global targets for HIV epidemic control by 2030.
- 2. Drug-resistant tuberculosis (TB) is a major contributor to antimicrobial resistance worldwide and is still a threat to public health. About half a million people do not respond to therapy because of TB-resistant strains globally.
- The introduction of WGS in AMV molecular TB surveillance is expected to improve the impact in all segments of the resistance and to replace the treatment in a timely manner.

Final goal: Expanded capacity for AMR surveillance in our country,

- systematic sampling of resistant isolates,
- quality control and
- collaborative networks will improve and extend reference databases of AMR organisms which will drive scientific research and lead to new science-driven engineering solutions that in turn can improve the use of WGS for AMR surveillance.

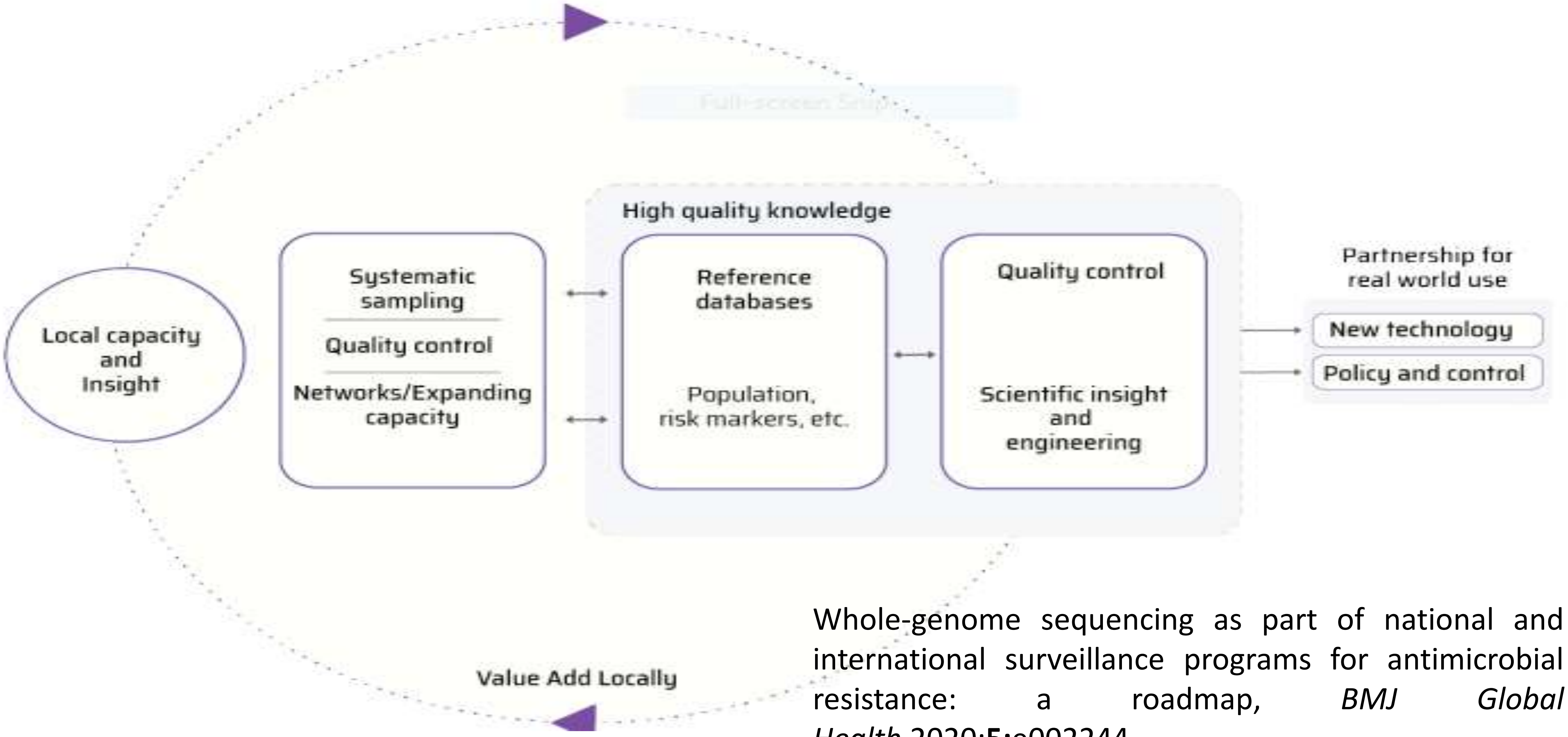
Together these three interlocking systems can lead to improved public health policy and AMR control and technological innovation.

- 1. Commitment of the country
- 2. Assessment of capacities, laboratories, infrastructure
- 3. Technical development
- 4. Preparing an action plan and standard operating practice SOP for WGS to ensure long-term sustainability

This steps should be flexibly adapted to each country's surveillance system

Establishment of WGS AMR surveillance

Virtuous circle of improved local capacity for WGS for AMR surveillance, improved reference databases and scientific research.



Whole-genome sequencing as part of national and international surveillance programs for antimicrobial resistance: a roadmap, *BMJ Global Health* 2020;**5:**e002244.

First step: commitment

- Most countries have obligations under International Health Regulations and Global AMR Strategies, including through WHO GLASS.
- Local and national commitment is important in establishing WGS as a complementary monitoring tool in building broader survival systems.
- The experiences of other countries that have already implemented WGS within their systems can be an important aspect of establishing WGS in our country.
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Second step

- Assessment of health system of how WGS could be included within existing surveillance systems for use
- Assessments of **laboratories, bioinformatics and supporting**
- WGS can be introduced as a complementary approach to existing phenotypic AST favoring retrospective sequencing,
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Third step: technical developmentneed for establishment of the technical capacity to sustain WGS for the long term

- including:
- capacity building
- leading research projects by providing resources and training,
- the development of **sampling frameworks** to address national public health and research needs,
- **data collection to** identify high-risk bacterial lineages and AMR genes,
- **Provision and integrated use of open-access tools for genomics analysis** and data visualization to understand the distribution of bacterial lineages.
- Technical guidance and tools are globally available to support the development of WGS for AMR surveillance.
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Third step

- In addition, open-access tools enable health professionals and policy-makers real time access to, and sharing of, genomic pathogen data for national AMR surveillance.
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- A starting point for technical development is to **establish a reference database including WHO priority pathogens** and **species of local importance.**
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- Capacity building is important step in support WGS establish surveillance system.
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- Workshops and training where the other countries' experiences (such is this meeting) could be heard would be highly useful for developing and maintaining this approach
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- An action plan covering laboratory, bioinformatics, data flow and financial management is useful to integrate WGS as part of the broader surveillance system.
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- Other country experience and support with SOPs and supply chain procedures, quality assurance (QA) and reporting processes, as well as ongoing capacity development, and processes for ongoing governance and financial sustainability would be of essential help

Fourth step:create a action plan and standard operating practice for SOPs WGS to ensure long-term sustainability

- An action plan covering laboratory, bioinformatics, data flow and financial management is useful to integrate WGS as part of the broader surveillance system.
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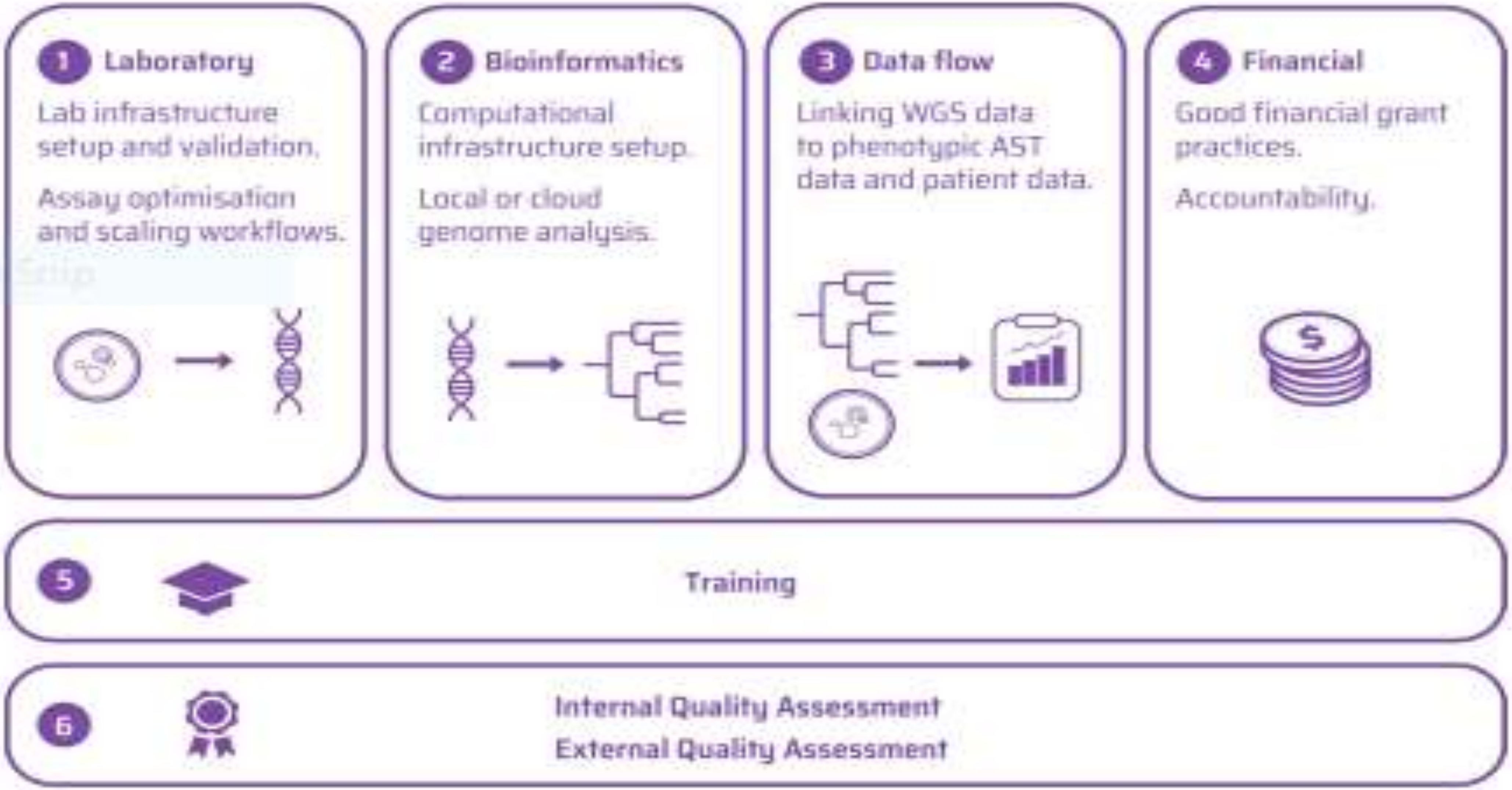
The challenges that we faced with are



Macedonian action plan for fighting antimicrobial resistance

- According to the recommendations of the World Health Organization (WHO) and the European Center for Disease Control and Prevention (ECDC) to control the spread of AMR, The Republic of Northern Macedonia became involved in organizing the control activities of AMR in 2008 with the appointment of a national coordinator for AMR and national coordinator for monitoring the consumption of antimicrobials in 2011 year.
- The appointment of the Multisectoral Commission for Control of AMR (MKAMR) within the Ministry of Health (MoH) enable joining global and European cross-sectoral efforts the problem of AMR.
- In order to provide a systemic response, ICAMR prepared it the **first National Strategy and Action Plan for Antimicrobial Control resistance for the period 2012-2016**,
- The strategy was adopted by the Government of R. Macedonia on April 26, 2011.
- The new strategy with an action plan to control AMR for the period until 2023, and is builds on the previous strategy and aligns with the WHO Global Action Plan on AMR
- Main goal of the National Strategy with action plan for control of AMR 2019-2023 year is an improvement of the health condition of the population in the North Republic Macedonia and through AMR control, preserving the effectiveness of antimicrobials funds.

The goal will be achieved by achieving the following strategic goals

- 1. Strengthen the monitoring of the resistance of microorganisms to antimicrobials and the provision of evidence-based data on AMR in human and veterinary medicine
- 2. Continuous monitoring of the consumption of antimicrobial drugs in humanity and veterinary medicine
- 3. Prevent and reduce the occurrence and control of the spread of infections
- 4. Rational use of antimicrobial drugs in human and veterinary medicine
- 5. Raising awareness and understanding of antimicrobial resistance in those who prescribe, issue and receive, ie use
- 6. Cooperation with institutions dealing with antimicrobial resistance as problem: ECDC, WHO, World Organization for Animal Health (OIE) and others.

- 7. Monitoring aintroduction of new diagnostic procedures and other interventions
- this is the key point at which the introduction of new technology can be extended and includedWGS
- nd participation in the development and detection of new antimicrobials,