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Highlights of existing WGS initiatives regarding CRE/CCRE







2016: "ECDC Expert opinion on whole genome sequencing for public health surveillance"

2016: "ECDC roadmap for integration of molecular and genomic typing into European-level surveillance and epidemic preparedness"

2019: "ECDC strategic framework for the integration of molecular and genomic typing into European surveillance and multi-country outbreak investigations"

- Background supporting WGS as the method with higher phylogenetic resolution in outbreak investigation
- WGS potential for *in silico* prediction of AMR phenotypes and typing
- Importance of harmonising bioinformatics analysis is noted, with focus towards phylogenetic analysis
- Data storage and exchange requirements + integration of sequence data with epidemiological and clinical data
- Five strategies to ensure WGS can be implemented without compromising surveillance, outbreak investigation or risk assessments during the transition period
- Specific proposals for integrating molecular/genomic typing methods into EU-level surveillance and epidemic preparedness relating to 12 priority pathogens
- Oriented surveillance would include, analyses of high-risk clones using strategies such as cgMLST typing schemes, pMLST typing of plasmids and prediction of antimicrobial resistance genes (ARGs) through sequence analysis against reference gene databases







2019: "Whole genome sequencing and metagenomics for outbreak investigation, source attribution and risk assessment of food-borne microorganisms"

 "WGS offers, in comparison to conventional typing methodologies, a more detailed outcome and new possibilities for food-borne outbreak detection/investigation, source-attribution and hazard identification. [...] The discriminatory power of WGS for pathogen characterisation is superior, compared to conventional molecular typing methods, leading to more robust case identification."

2019: "EFSA and ECDC technical report on the collection and analysis of whole genome sequencing data from food-borne pathogens and other relevant microorganisms isolated from human, animal, food, feed and food/feed environmental samples in the joint ECDC-EFSA molecular typing database"

- Requirements of such database: grouped in data collection (submission, storage and sharing), data analysis (sequence read data quality, genome assembly, phylogenetic inference (further categorised into whole and core genome MLST, SNP analysis, k-mer-based distance estimates and comparing phylogenetic relationships), strain nomenclature and genome characterisation, general requirements and infrastructure.
- Each requirement was used to evaluate existing bioinformatics tools or initiatives, concluding that no existing tool was able to properly respect all requirements and that a combination of solutions should be considered.

2021: "EFSA statement on the requirements for whole genome sequence analysis of microorganisms intentionally used in the food chain"

Several recommendations can potentially be applied to public health: the suggestion to adapt a threshold of maximum 500 contigs for *de novo* assembled bacterial genomes, with a total size within 20% of deviation from the expected genome size for the respective species, and to perform detection of ARGs against at least two reference gene databases accepting a minimum of 80% identity and 70% coverage.



CURRENT EUROPEAN UNION AND INTERNATIONAL GUIDANCE





2018: "Landscape paper on whole genome sequencing for foodborne disease surveillance" 2020: "Global Antimicrobial Resistance and Use Surveillance System (GLASS) document on whole-genome sequencing for surveillance of antimicrobial resistance"

- Essential to standardize protocols, data analysis pipelines and reporting guidelines before such an approach can be implemented at local, national or international level
- Ensuring that it is possible to compare new WGS data with older results acquired through different protocols.
- The proper infrastructure has to be developed both in terms of necessary physical locations, hardware and data management but also training of the respective professionals across the sectors of public, veterinary and human health. The organization of these sectors and communication with regulatory agencies should be reviewed.
- Legislation needs to be adapted to include acceptance of WGS results instead or in combination with the results of current methods used for surveillance and diagnostics, which also implies the creation and validation of normalized interpretative criteria for WGS results.



TBA: ISO/DIS 23418 standard "Microbiology of the food chain – Whole genome sequencing for typing and genomic characterization of foodborne bacteria – General requirements and guidance"







Agreement on necessary requirements:

• prediction of clinically and epidemiologically relevant microbial phenotypes

antigenic profile, AMR and virulence, including identification of determinants encoded in the accessory genome and mobile genetic elements

- phylogenetic analysis
- well defined QC parameters
- integration of sequence data with epidemiological and clinical data
- database for the collection and analysis of WGS data + proper management









CGE: Centre for Genomic Epidemiology GMI: Global Microbial Identifier VEO: Versatile Emerging Infectious Disease Observatory



NETWORKS FOCUSING ON OR INCLUDING CRE/CCRE



Network	Organization	Sec	ctor	Main target audience	Type of guidance/support
EARS-Net	ECDC	*]	National AMR surveillance laboratories and clinical microbiology laboratories	Monitoring of AMR, providing guidelines, designing recommendations
EURGen-Net	ECDC	*		NRLs for AMR	Monitoring of AMR, providing guidelines, designing recommendations
EpiPulse	ECDC	*		National PH authorities	Monitoring of AMR, providing guidelines, designing recommendations
EURL-AR	EC		101 175	NRLs	Method development, laboratory and bioinformatics training, proficiency testing
EURL-VTEC	EC		101 100	NRLs	Method development, laboratory and bioinformatics training, proficiency testing
ENGAGE	EC	ŧ	10 17 5	All	Method development, networking, development of bioinformatics solutions, training
COMPARE	EC	*	10 17 5	All	Method development, networking, development of bioinformatics solutions, training, proficiency testing
VEO	EC	Ŷ	10 1 7 7	All	Method development, networking, development of bioinformatics solutions
NARMS	US CDC	Ŷ	10 17 5	PH laboratories and food authorities	Monitoring of AMR, providing guidelines, designing recommendations
AMD	US CDC	ŧ	10 17 5	PH laboratories and food authorities	Method validation, networking, development of bioinformatics solutions
PulseNet	US CDC	*	10 17 5	PH laboratories and food authorities	Monitoring of AMR, providing guidelines, designing recommendations, laboratory and bioinformatics training
GenomeTrakr	US CDC		101 100	PH laboratories and food authorities	Method development, networking, development of bioinformatics solutions
GMI	US CDC, DTU, FDA	ŧ	10 17 5	All	Method development, networking, development of bioinformatics solutions, proficiency testing
GLASS	WHO	•		National AMR surveillance laboratories and clinical microbiology laboratories	Monitoring of AMR, providing guidelines, designing recommendations





CONCLUSIONS



- Recommendations are similar regardless of setting (e.g. PH vs. food)
 - Importance of harmonization
 - Importance of data management infrastructures
- Several networks focused on training
 - Many training materials available
 - Potential to share and compare results with other national laboratories/other areas
- · Standardization on the way
 - Almost agreement on QC parameters
 - Almost ISO standard (!! foodborne)
 - EURGen-RefLabCap + FWD AMR-RefLabCap

