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The present and future in antimicrobial resistance surveillance

- Rene Hendriksen

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Global situation of antimicrobial resistance

"**Antimicrobial resistance is a crisis** that must be managed with the **outmost urgency**…..

….**Antimicrobial resistance threatens the very core of modern medicine** and the sustainability of an effective, global public health response to the enduring threat from infectious diseases…

…**Without harmonized and immediate action on a global scale, the world is heading towards a post-antibiotic era in which common infections could once again kill**"

Dr Margaret Chan
Director-General (former)
World Health Organization

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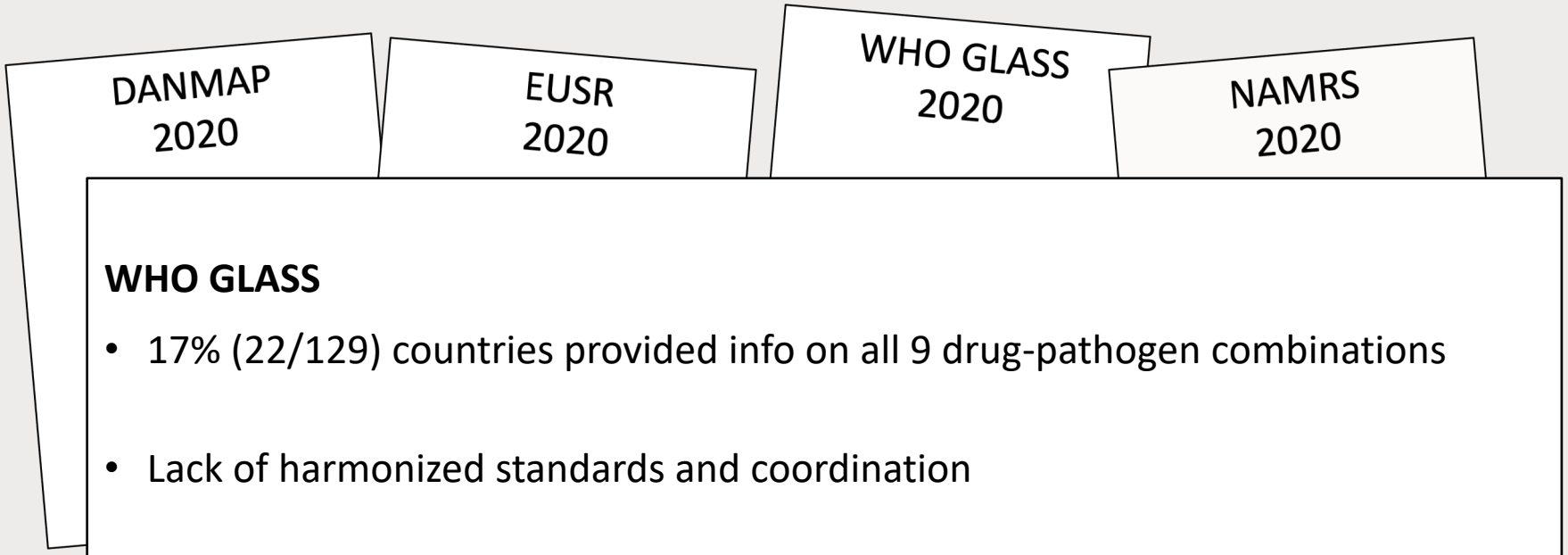
Surveillance systems in place



WHO GLASS

- 17% (22/129) countries provided info on all 9 drug-pathogen combinations
- Lack of harmonized standards and coordination
- Country data, when available, not shared with national bodies
- Limited information on impact of antibacterial resistance on humans
- As of today, 22 March 2019, 75 countries participate in GLASS

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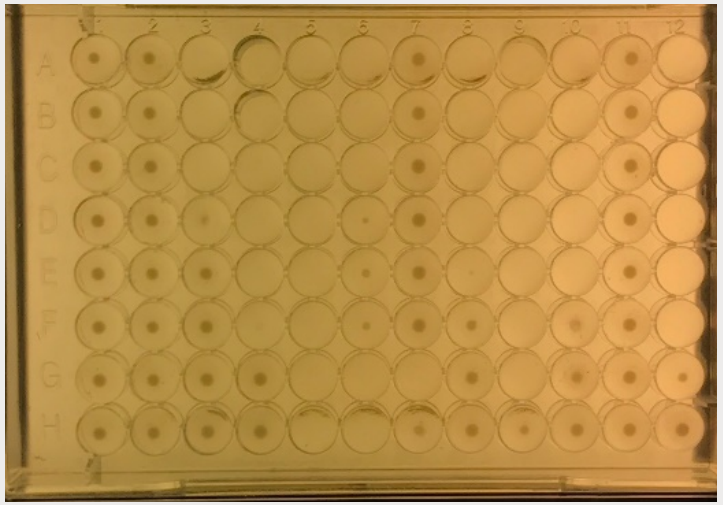
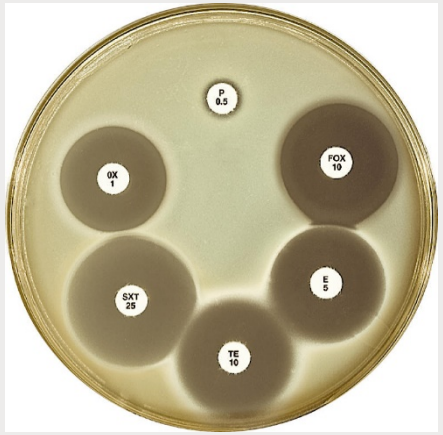
Phenotypic antimicrobial susceptibility testing - Methodology

- Well-tested standardized approaches
- Most variables harmonized e.g. drug panels, MIC, ECOFFs etc.
- Used to infer resistance (S/I/R)





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Phenotypic antimicrobial susceptibility testing - Deviation level based on PTs

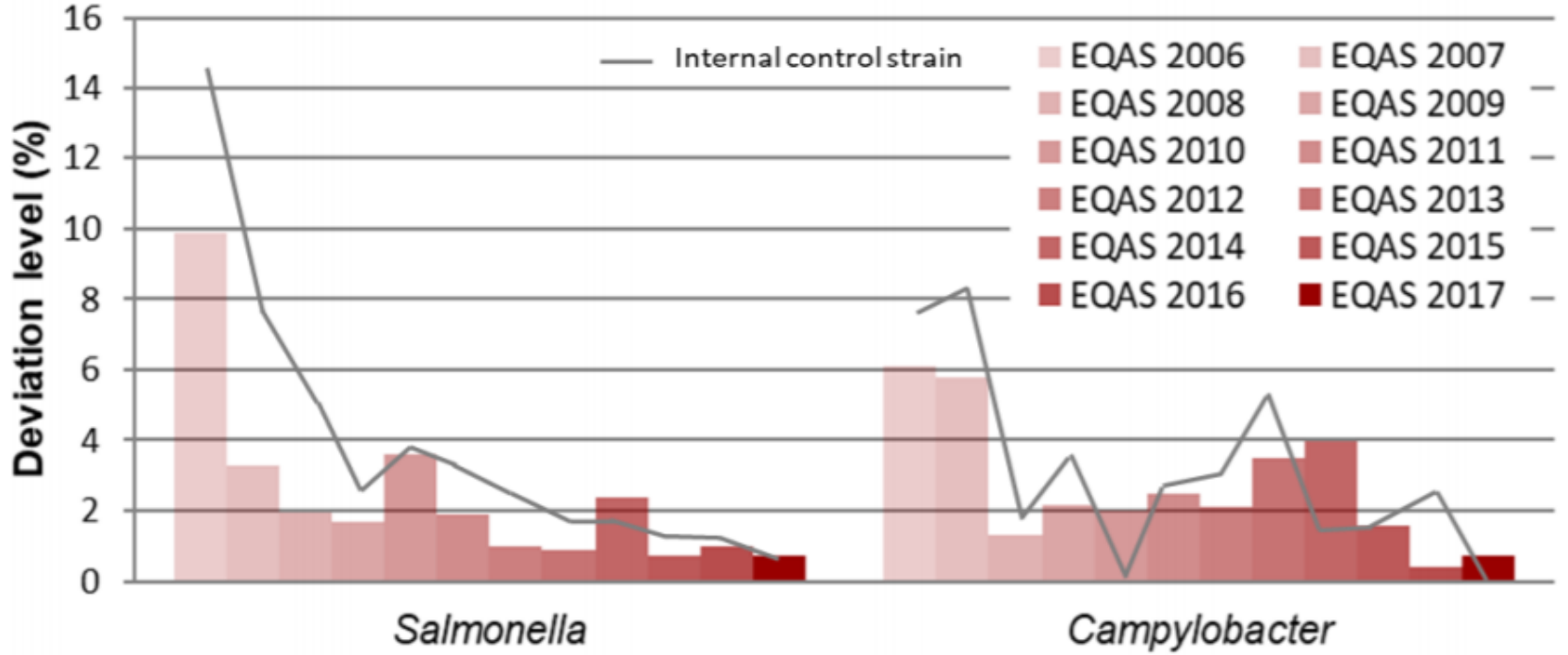

Figure 2: A comparison between the EURL-AR EQAS's since 2006, showing the total percentage of deviations for antimicrobial susceptibility testing performed by participating laboratories.

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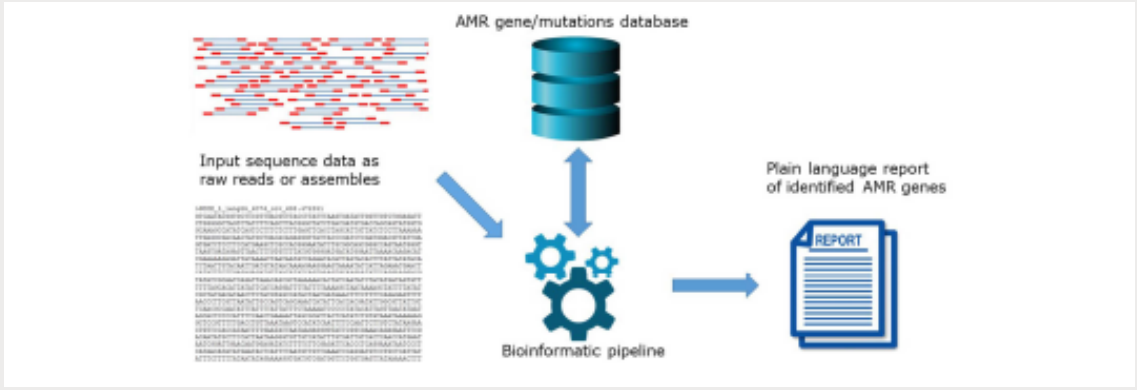
What is Whole Genome Sequencing?

- WGS is a molecular biology tool used to generate the complete DNA sequence of an organism
- Provide better understanding of the mechanisms of resistance and other markers incl. -the relatedness of strains for investigating the emergence and spread of AMR
- Offers a vast amount of information and the highest resolution for molecular subtyping of pathogens



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Paradigm shift in surveillance – "Biggest revolution since Pasteur"

"It is likely that in 5 to 10 years, all clinical microbiological laboratories will have a DNA sequencer in use - the costs for a complete bacterial genome sequence might be less than 50 Eur/ US$

What do we have in place?

The capacity to exchange – and manage - large data quantities over web-based systems has likewise increased dramatically over recent years

Enabling the potential creation of global databases consisting of DNA-codes of all relevant microbiological strains"

Source: Statement from the international expert meeting on GMI 1-2 September 2011 in Brussels, Belgium.

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Use of WGS for AMR identification

Figure 1. Annual numbers of publications on use of WGS for AMR surveillance of GLASS priority pathogens

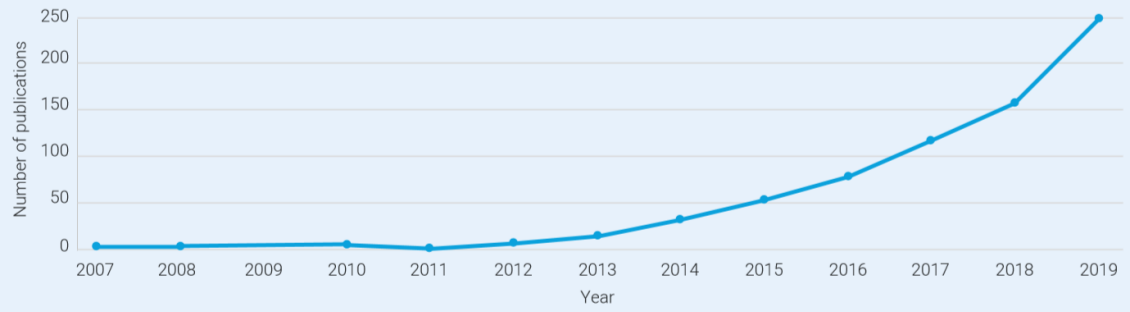
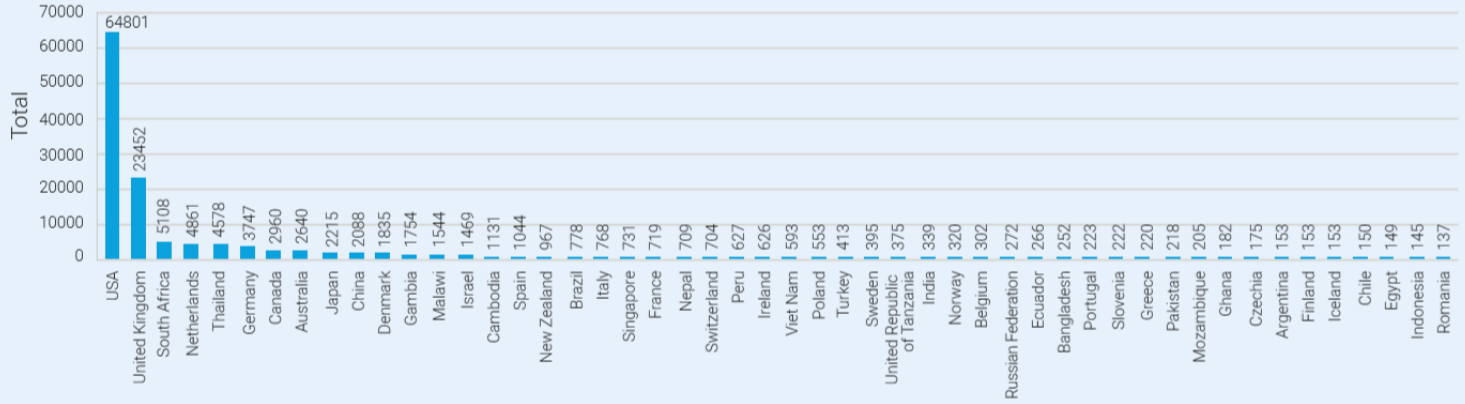


Fig. 2. Numbers of sequenced isolates of GLASS priority pathogens by country of origin in the European Nucleotide Archive.



Only the first 50 countries in terms of isolate numbers are shown. Numbers above bars are the numbers of sequenced isolates. The Archive contained 141 210 sequences of GLASS priority pathogens from 126 countries as of July 2019.

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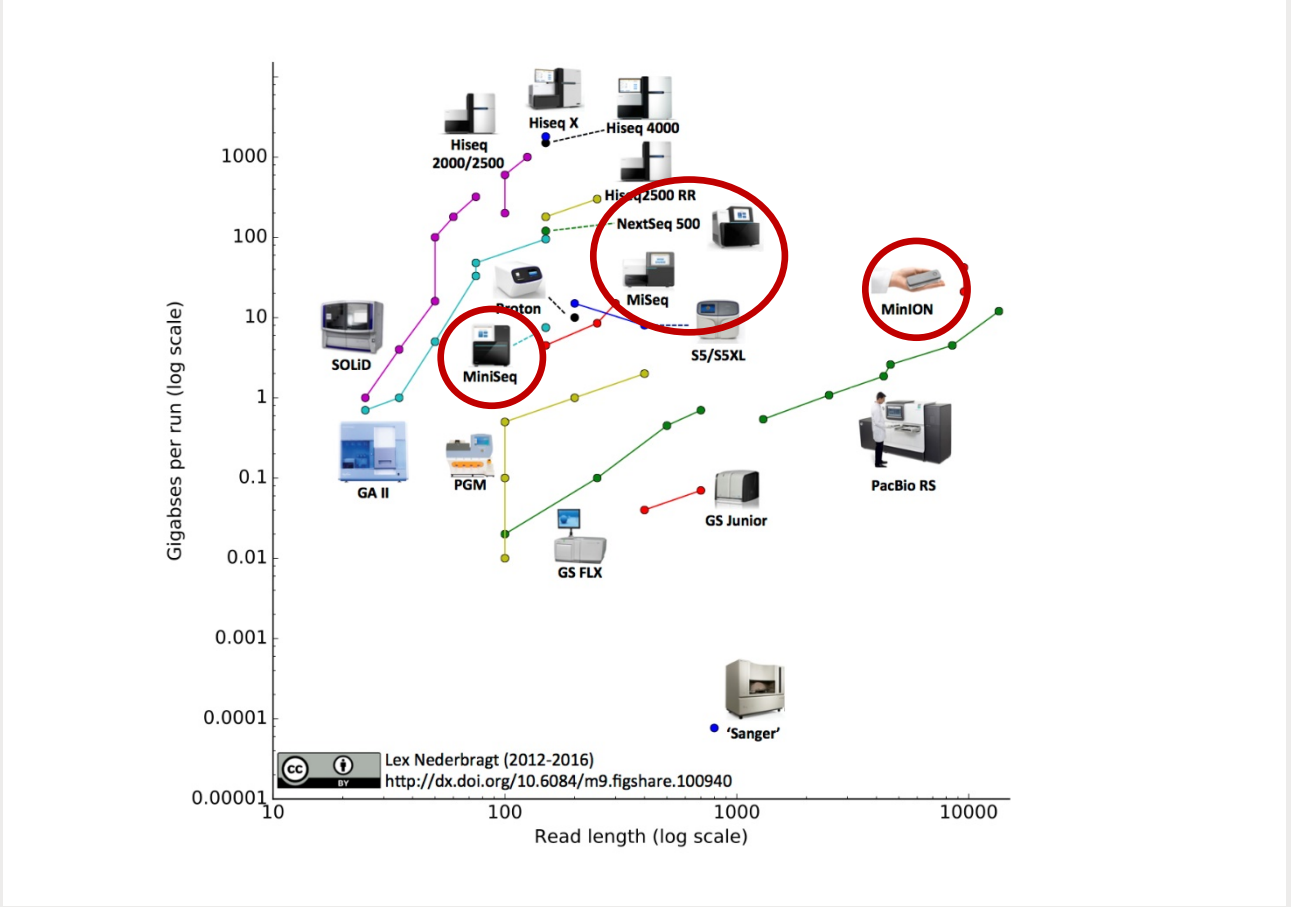
Sequencing platform development



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Tools to predict antimicrobial resistance genes

- App. 47 resources for *in silico* prediction of AMR determinants exists
- System features differ widely as to, in- and out-put format
- Web-based vs commandline (GitHub)
  - Shield end-user from complexities
- Open access vs commercial available
- Computing time

ResFinderFG
Galileo AMR (MARA, RAC)
LREfinder
MUBII-TB-DB
Mykrobe
TBDReaM
PointFinder
SCCmec Finder
U-CARE
ARGDIT
ARG-miner
ResFinder (DTU CGE)
SRST2
ARG-ANNOT
ARIBA
CARD
Kmer resistance (DTU CGE)
MEGARes (AMRplusplus)
NCBI AMRFinder

Hendriksen et al., Front Public Health. 2019

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Building Global Capacity – CGE

Overview of Services

Workflows

Bacterial Analysis Pipeline (Batch Upload)

Typing

KmerFinder
CCMetagen
SpeciesFinder
MLST
PlasmidFinder
pMLST
cgMLSTFinder
SerotypeFinder
FimTyper
CHTyper
spaTyper
PAst
SCCmecFinder

Phylogeny

CSI Phylogeny
NDtree
Evergreen
snpTree (Out of order, use CSI Phylogeny or NDtree)
TreeViewer

Phylogeny

CSI Phylogeny
NDtree
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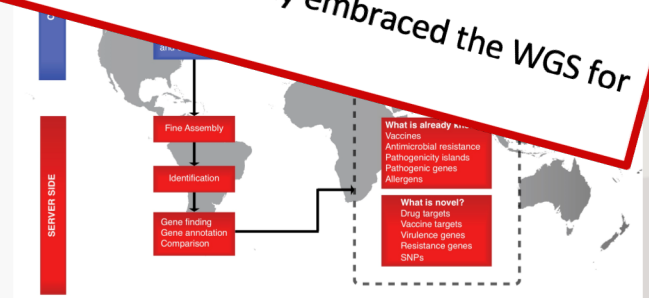
snpTree (Out of order, use CSI Phylogeny or NDtree)
TreeViewer

Metagenomics

CCMetagen

- More than 2.5 million (>2.000 genome / day) submissions from +150 countries
- Leapfrog the Whole Genome Sequencing technology to LMIC
- Ensure global harmonization among those countries already embraced the WGS for surveillance and outbreak detection



http://cge.cbs.dtu.dk/services/all.php

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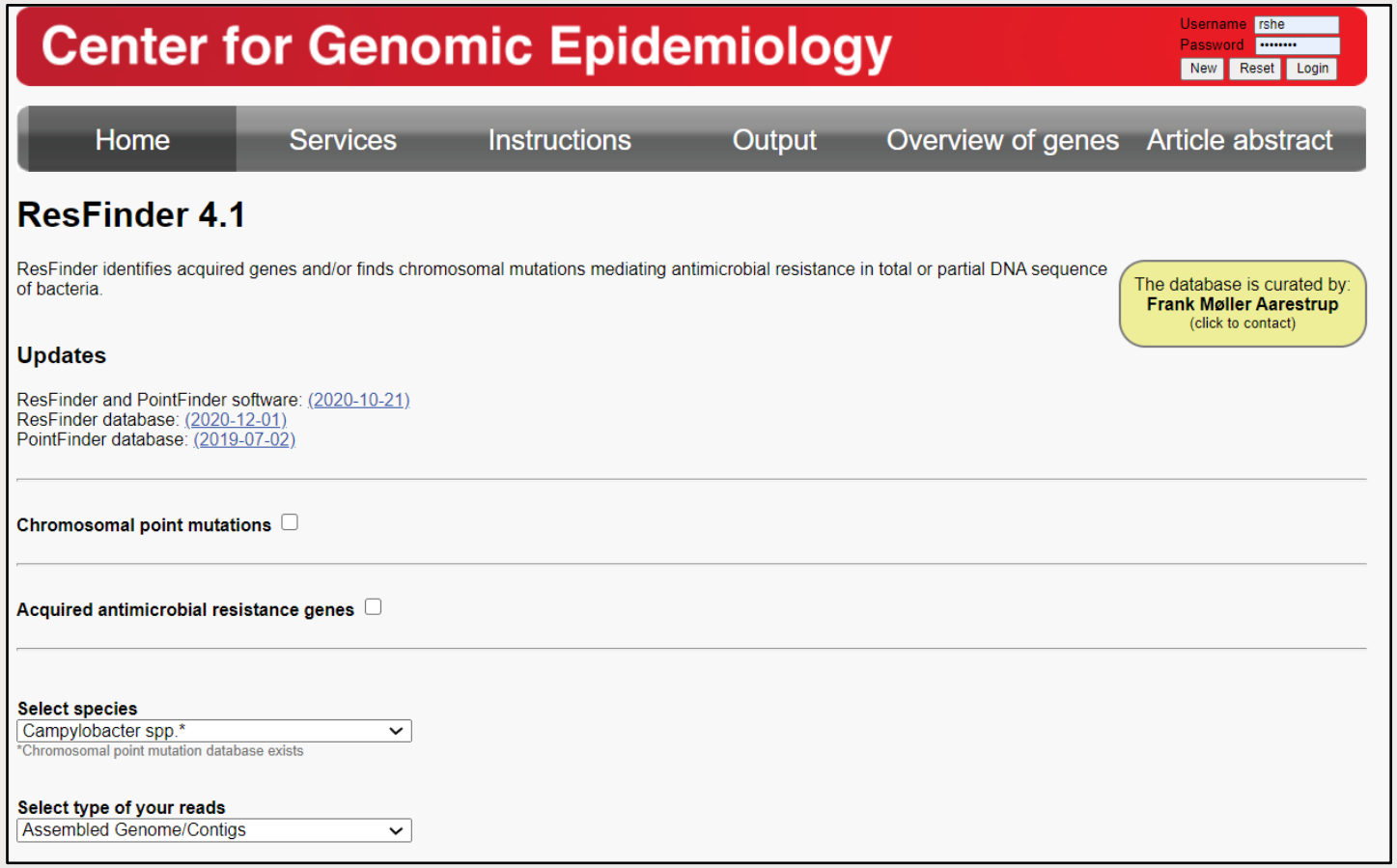
Tools to predict antimicrobial resistance genes



https://cge.cbs.dtu.dk/services/ResFinder/ar

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Tools to predict antimicrobial resistance genes

escherichia coli | complete

Antimicrobial	Class	WGS-predicted phenotype	Genetic background
temocillin	beta-lactam	No resistance	
cefotaxime	beta-lactam	Resistant	blaCMY-2 (blaCMY-2_X91840)
ciprofloxacin	fluoroquinolone	Resistant	gyrA (S83L)
cefotaxime-clavulanic acid	NA	NA	Not in database
ceftazidime	beta-lactam	Resistant	blaCMY-2 (blaCMY-2_X91840)
sulfamethoxazole	folate pathway antagonist	No resistance	
imipenem	beta-lactam	No resistance	
tigecycline	tetracycline	No resistance	
gentamicin	aminoglycoside	No resistance	
cefepime	beta-lactam	No resistance	
chloramphenicol	phenicol	No resistance	
ceftazidime-clavulanic acid	NA	NA	Not in database
meropenem	beta-lactam	No resistance	
tetracycline	tetracycline	No resistance	
colistin	polymyxin	No resistance	
ertapenem	beta-lactam	No resistance	
nalidixic acid	fluoroquinolone	Resistant	gyrA (D87N)
cefoxitin	beta-lactam	Resistant	blaCMY-2 (blaCMY-2_X91840)
azithromycin	macrolide	No resistance	
trimethoprim	folate pathway antagonist	No resistance	
ampicillin	beta-lactam	Resistant	blaCMY-2 (blaCMY-2_X91840)

Download phenotype table (txt) | Download species specific phenotype table (txt)

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Phenotype / genotype concordance

- High concordance (> 96%) between acquired resistance genes / mutations and MIC
- High levels of sensitivity (>87%) and specificity (>97%) have been observed depending of the species analysed

Pathogen	No. of pathogens	AST method	No. of antimicrobials	Bioinformatic tool	Sequencing data	Concordance	Sensitivity	Specificity	Comment	Reference
S. Typhimurium E. coli E. faecalis E. faecium	49 48 50 50	MIC	17 14	ResFinder	Assembled, Velvet	99.74%			Disagreement: 7 isolates: 6 E.coli to SPEC	Zankari etal., 2013
E. coli (ESBL) K. pneumonia (ESBL)	74 69	DD	7	BLASTn, selected panel	Assembled, Velvet		96%	**97%**	VM rate: 1.2%/ M rate: 2.1%	Stoesser et al., 2013
S. aureus	501	DD/ MIC (Vitek)	12	BLASTn, selected panel	Assembled, Velvet		97%	99%	VM rate: 0.5%/ M rate: 0.7%	Gordon NC et al., 2014
C. jejuni C. coli	32 82	MIC	9	BLASTx	Assembled, CLC	99.2%			Lower concordance to Gen, Azi, Clin, Tel	Zhao et al., 2016
S. enterica	104 536	MIC	14	ResFinder/ ARG-ANNOT/ CARD/ BLAST	Assembled, CLC	99.0%	99.2% 97.6%	99.3% 98.0%	Lower concordance to aminoglycosides / β-lactams	McDermott et al., 2016
E. coli K. pneumonia P. aeruginosa E. cloacae	31 24 22 13	MIC	4	Custom DB based on ARDB/ CARD/ β-lactamase allelles			**87%**	98%	Neg. predictive value: 97% Pos. Predictive value: 91%	Shelburne et al., 2017
S. enterica E. coli C. jejuni	50 50 50	MIC	4 6 4	ResFinder/ PointFinder	Assembled, SPAdes	98.4%			Disagreement: 2/2 C.jejuni to FQ/ERY 5 E.coli to COL (pmrB)	Zankari etal., 2017
E. faecalis E. faecium	97 100	MIC	11	ResFinder/ NCBI Pathogen DB/ BLAST	Assembled, CLC	**96.5%**				Tyson et al., 2018
S. aureus	501 491 397	DD / MIC MIC	12	GeneFinder/ Mykrobe/ Typewriter	fastq / assembled, BLAST	98.3%			Disagreements: 0.7% predicted resistant 0.6% predicted susceptible	Mason et al., 2018
M. tyberculosis	10.209	MGIT 960	Isoniazid Rifampin Ethambutol Pyrazinamide	Cortex	Assembled	89.5%			97.1%/ 99.0% predicted R/ S 97.5%/ 98.8% predicted R/ S 94.6%/ 93.6% predicted R/ S 91.3%/ 96.8% predicted R/ S	Walker et al., 2018
H. pylori	140	MIC (E-test)	5	ARIBA	fastq	99%			Phenotype issues to metronidazole	Lauener et al., 2019

Hendriksen et al., 2019 https://www.frontiersin.org/articles/10.3389/fpubh.2019.00242/full

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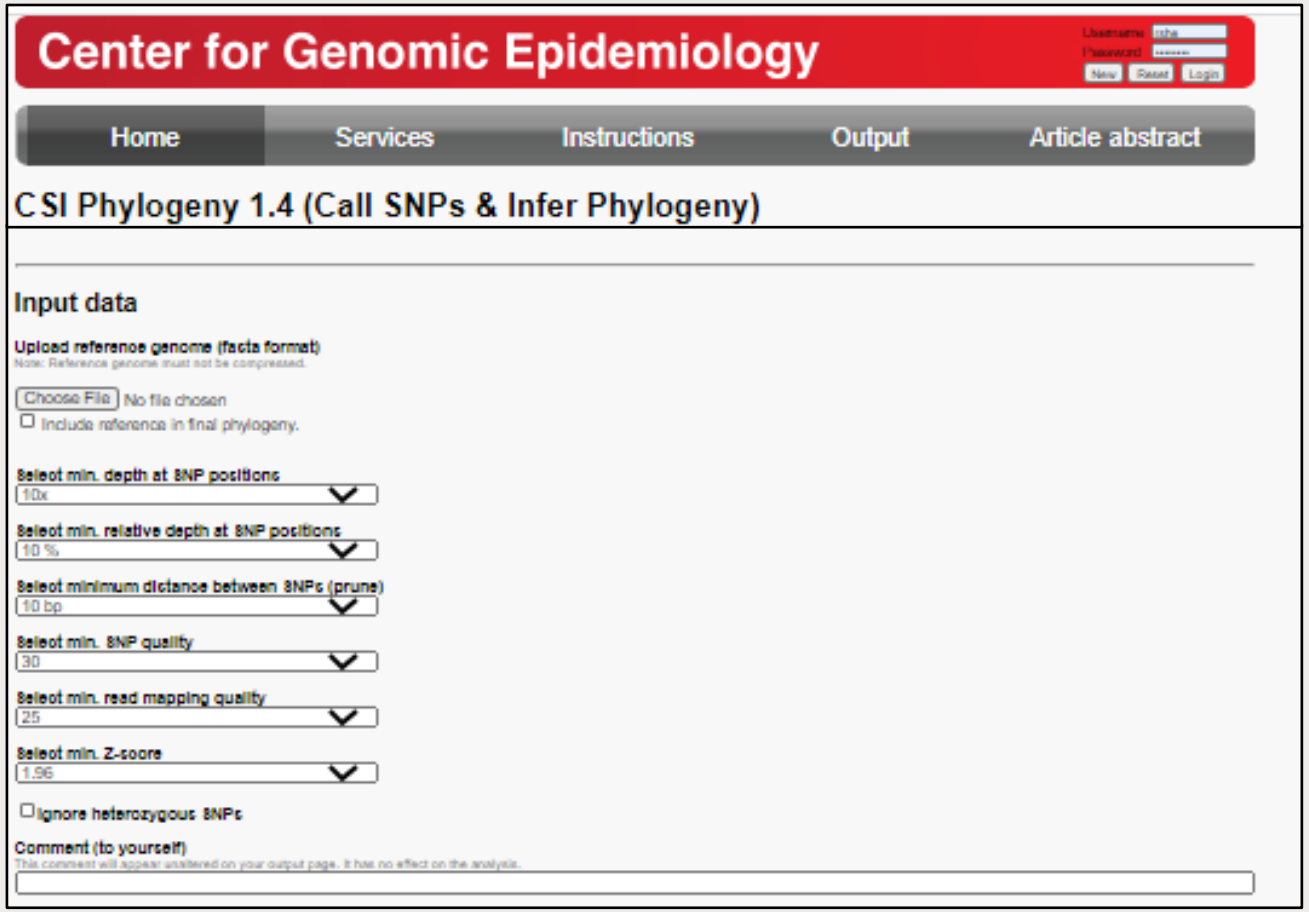
Tools to perform phylogeny – CGE



https://cge.cbs.dtu.dk/services/CSIPhylogeny/

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AMR characterization according to surveillance objectives

Objectives that can be fully met with phenotypic methods:

- Trends of AMR rates
- Assessment of frequency of AMR infections
- Data to inform national list of essential antimicrobial medicines
- Treatment guidance

WGS as complement of phenotypic methods:

- To understand the genetic basis of AMR mechanisms and differentiate phenotypically identical isolates with the same AST profile.
- To allow the location of AMR determinants on the bacterial chromosome or in plasmids, which provides valuable information on the pathways of AMR spread.
- To facilitate linkages during the early investigation phase of outbreaks.

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Potential uses of WGS for AMR surveillance

- **Local uses of WGS for AMR surveillance include:**
  - detection of known AMR mechanisms
  - identification of novel AMR mechanisms, with phenotypic AST data and characterization as e.g. plasmid-mediated or clonal
  - analysis of an outbreak at a single centre, such as a hospital.

- **Local, subnational or national uses of WGS for AMR surveillance include:**
  - comparison of several genomes from different sites
  - analysis of local or subnational transmission networks
  - tracing sources of local or regional outbreaks

- **International uses of WGS for AMR surveillance include:**
  - monitoring of pathogen populations
  - detection of high-risk AMR clones
  - assessment of the impact of interventions
  - detection of multi-country outbreaks

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Current limitations of WGS for AMR surveillance

- WGS technologies require substantial initial and sustained financial investments
- Procurement of instruments and consumables are a bottleneck
- Sequencing and bioinformatics are not part of the general knowledge or training of staff in laboratories in LMIC, and investment in training and continuous education of staff must be secured
- Standard operating procedures, QA protocols and evidence-based guidelines should be developed for use of WGS in AMR surveillance
- Data-sharing is not currently standard practice

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Requirements needed to embark on WGS

- Timing of Introduction
- Infrastructure Requirements for whole-genome sequencing
  - Laboratory Capacity
  - Bioinformatics and computational capacity – open / proprietary?
- Quality assurance, quality control and international standardization
  - Crucial requirements for international QC
  - Bioinformatics QC
- Procurement
- Training
- Data collection, sharing and storage of sequence and metadata

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In summary

- WGS is rapidly entering diagnostic and public health, with near real time data generation
- WGS is bringing the opportunity to countries to enhance laboratory activities for surveillance and research
    - introduction of any new technology should consider the existing available resources and country needs
- WGS is a realistic alternative to conventional AMR surveillance
    - Powerful way to determine prevalence and differences of all genes
- Advantages to detect all known genes including AMR are an asset to understand the spread of AMR and taking action
- A need for better infrastructure and agreements to meet the coming demand

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Thank you

www.antimicrobialresistance.dk

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The views expressed do not necessarily reflect the UK Government's official policies.