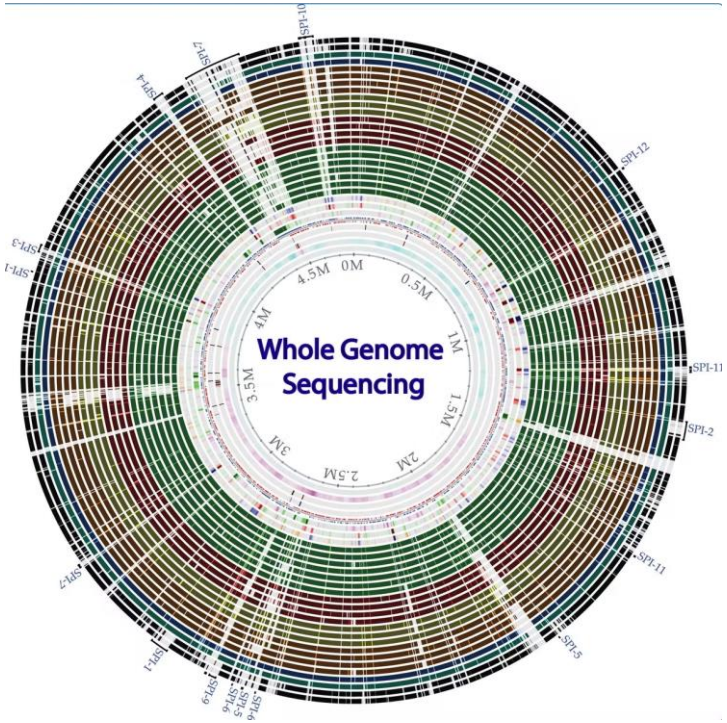
HOSPITAL SOUTH WEST JUTLAND , RESEARCH UNIT OF
CLINICAL MICROBIOLOGY

EURGen-RefLabCap workshop on capacity building

WGS from the perspective of the clinical laboratory



John Coia, Clinical Professor, MD, Department of Clinical Diagnostic, Hospital South West Jutland , University Hospital of Southern Denmark and Research Unit of Clinical Microbiology, Department of Regional Health Research, University of Southern Denmark
29 June 2022

Background

- Lead Consultant microbiologist at the Hospital of South-West Jutland (SVS) and Professor in Clinical Microbiology at Institute for Health Research, SDU
- Consultant microbiologist for over 25 years in Scotland responsible for diagnostic laboratory services in Edinburgh and Glasgow
- Former Director for Scottish Reference Laboratory facilities in Glasgow (including AMR reference laboratory)






Region Syddanmark

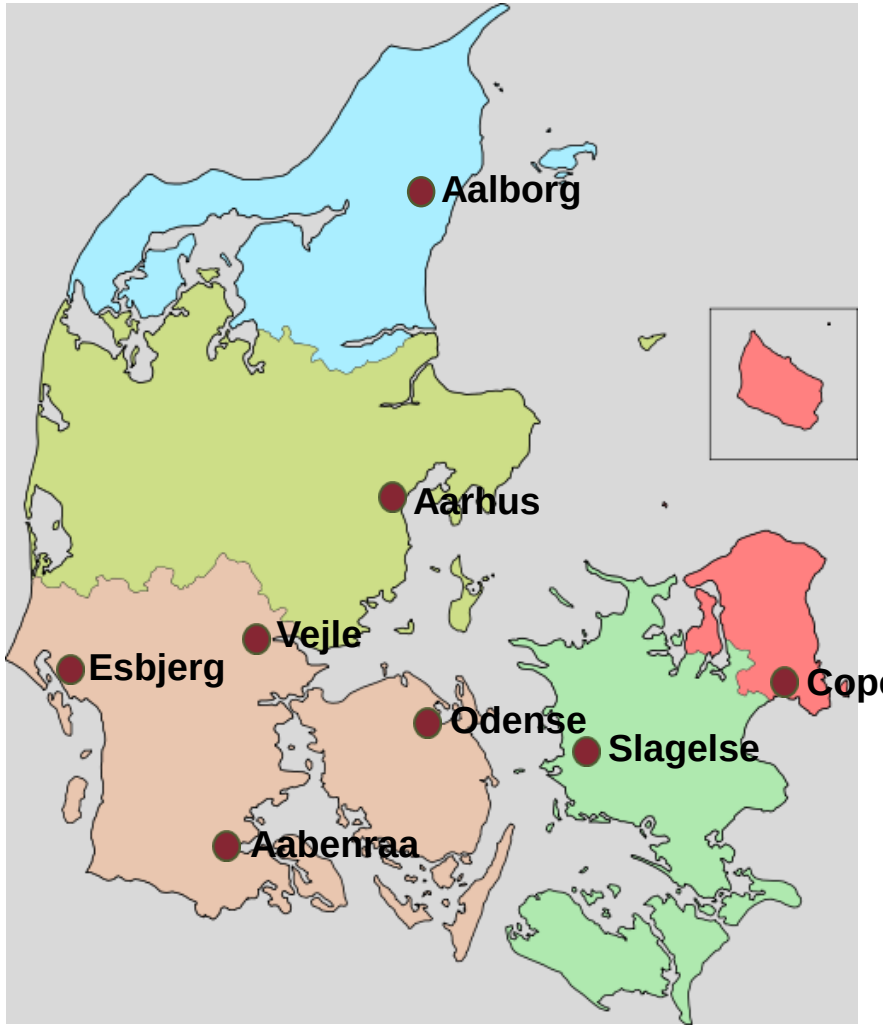
Overview

1. How do the clinical microbiology laboratories in hospitals in Denmark currently work with the national reference laboratory for AMR?
2. How do the clinical microbiology laboratories use the reports and advice from the NRL?
3. Other uses of WGS from the perspective of the clinical laboratory

How do the clinical microbiology laboratories in hospitals in Denmark currently work with the national reference laboratory for AMR?

Organisation of Clinical Microbiology in Denmark

- Danish population is 5.9 million
- There are 10 human diagnostic clinical microbiology laboratories (KMAs)
- Each employs a mixture of medically-qualified specialists in clinical microbiology, scientists, technical staff, and administrative staff
- Responsible for the provision of the majority of diagnostic microbiology services for human patients
- SSI in Copenhagen provides specialist diagnostic services, as well as typing and epidemiological, and national surveillance and national infection prevention and control guidance



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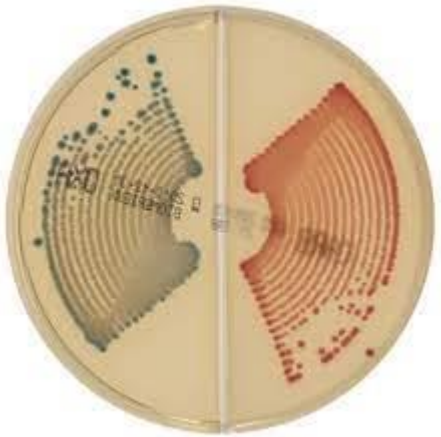
SDU

Role of the diagnostic laboratories

- Clinical microbiology laboratories are responsible for initial detection, isolation and antimicrobial susceptibility testing of organisms isolated from patient samples from hospitals and GPs
  - Suspected infection
  - Screening samples
- Detection/isolation of pathogens uses a mixture of conventional culture techniques and/or molecular methods
- The individual laboratory has responsibility for choice of methodology. All of the human clinical diagnostic laboratories participate in EQA programmes, and have quality systems in place
- Identification of organisms is performed predominantly by MALDI-TOF, and initial resistance determination is mainly based on disk diffusion according to NORDICAST criteria.
- Initial identification and resistance testing can be augmented by other phenotypic and molecular methods

Carbapenem resistant organisms

Screening Test
Previously positive
Household contact
Transfer from another hospital in Scandinavia
Hospital Treatment outside of Scandinavia in the last 6 months
Lived outside Scandinavia in the last 6 months and received antibiotic treatment
Lived under harsh conditions in the last 6 months (war zone/refugee etc)



Screen with chromogenic agar

Isolate from clinical infection

Phenotypic sensitivity testing (Disk diffusion and E-test)

MALDI-ID

Region Syddanmark

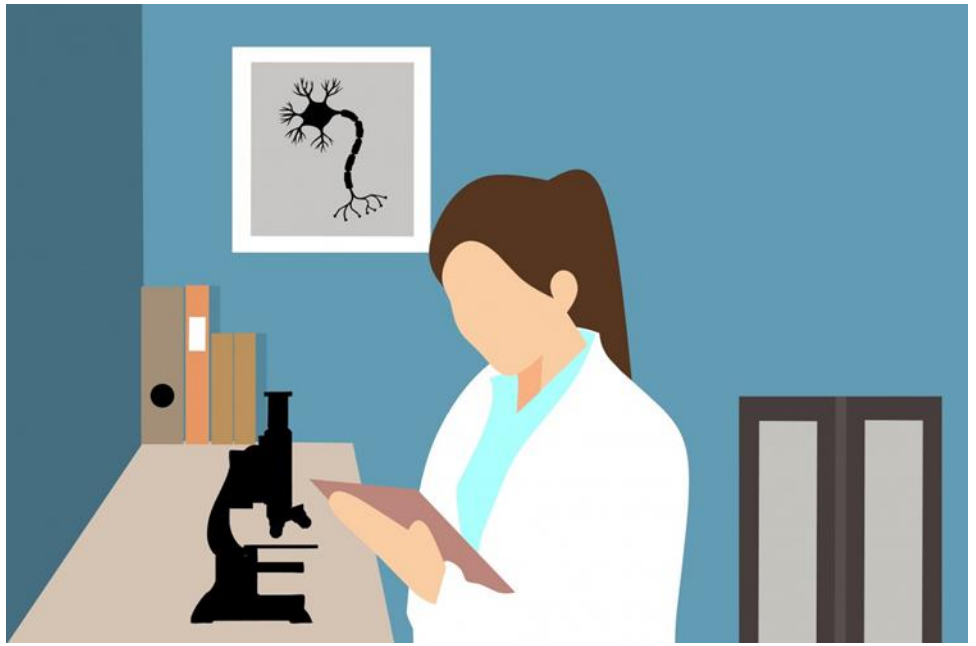
Criteria for referral of isolates

Organism	Isolate sent to NRL	Numbers recorded in national surveillance
Carbapenem-resistant (Enterobacteriaceae, *Pseudomonas* spp, *Acinetobacter* spp.)	All first isolates, >1 year since last isolate, Altered ID, Altered resistance phenotype, Altered carbapenamase type	Yes

Support from the NRL

- Confirmation of ID and presence of carbapenemase
- Identification of carbapenemase genes
- Detection of targets missed by local KMA methods
  - CARBA 5 NG: KPC, OXA-48 like, VIM, IMP, NDM
- Molecular typing by WGS
- Additional antimicrobial testing
- Advice and support
  - Criteria for sending isolates to the NRL
  - Advice on investigation of outbreaks

How do the clinical microbiology laboratories use the reports and advice from the NRL?



Criteria for referral of isolates

Organism	Isolate sent to NRL	Numbers recorded in national surveillance
Carbapenem-resistant (Enterobacteriaceae, *Pseudomonas* spp, *Acinetobacter* spp.)	All first isolates, >1 year since last isolate, Altered ID, Altered resistance phenotype, Altered carbapenemase type	Yes
VRE	All first isolates, >1 year since last isolate	Yes
MRSA	All first isolates, >1 year since last isolate, Altered resistance phenotype	Yes
Clostridium difficile	Suspected outbreak, Suspected ribotype O27, All first isolates per patient in April/October	Yes (toxin-positive)
Vibrio spp.	All first isolates/episode	Yes
Salmonella spp.	All first isolates/episode	Yes
STEC/EPEC/ETEC/EIEC	All first isolates/episode	Yes
Yersinia spp.	All first isolates/episode	Yes
Listeria spp.	All first isolates/episode	Yes
Campylobacter spp.	Not routinely sent	Yes

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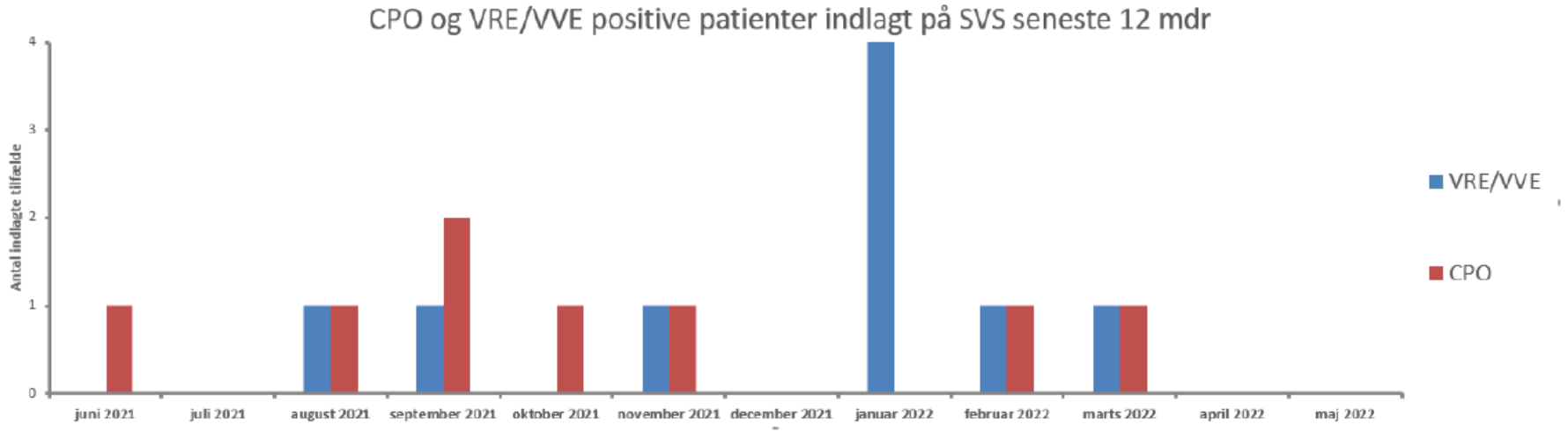
Example from the annual Infection Prevention and Control report from SVS for 2021/2022

12.4 Resistente mikroorganismer

Organisatorisk enhed	Type	Antal nye tilfælde blandt indlagte patienter	Hele SVS sidste 12 måneder
SVS	CPO	0	8
SVS	VRE/VVE	0	9



Fodnote:
CPO tilfælde i juni 2021 og august 2021 er fundet i forbindelse med screening ved overflytning fra andet sygehus.
Det ene CPO tilfælde i september findes tilfældigt hos en patient med hyppige kontakter med sundhedsvæsenet - ved efterfølgende smitteopsporing fandtes ingen spredning. Det andet CPO tilfælde og VRE tilfælde i september 2021 blev fundet ved samme patient, i forbindelse med screening ved overflytning fra udenlandsk sygehus.
CPO tilfælde i oktober blevet fundet tilfældigt i forbindelse udredning (en klinisk prøve) ved indlæggelse. Patienten har op til indlæggelsen opholdt sig 3 mdr. i udlandet.
CPO tilfælde i november 2021 fundet i forbindelse med screening ved overflytning fra andet sygehus.
To af de fire VRE tilfælde i januar 2022 er fundet i forbindelse med screening ved overflytning fra andet sygehus. De to øvrige tilfælde er fundet på Intensiv afsnit (hos udenlandsk patient med sekundær smitte til en anden intensiv patient).
CPO og VRE tilfælde i februar 2022 er fundet hos samme patient i forbindelse med screening ved overflytning fra andet sygehus.
CPO og VRE tilfælde i marts 2022 er fundet hos to forskellige patienter i forbindelse med screening ved overflytning fra andet sygehus.

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SDU

Carbapenamese-resistant *Acinetobacter baumanii* EpiLinx outbreak report

Dato

Udbrudsrapport

A. baumannii ST1816 CT2045 OXA-23 Esbjerg Sygehus

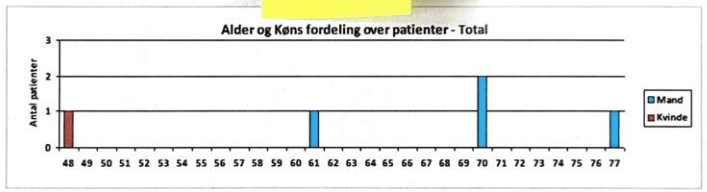
Udbrudsnavn og KURS-nr.

Ab0010_ST1816_CT2045_OXA-23 KURS ID 1067

Demografi

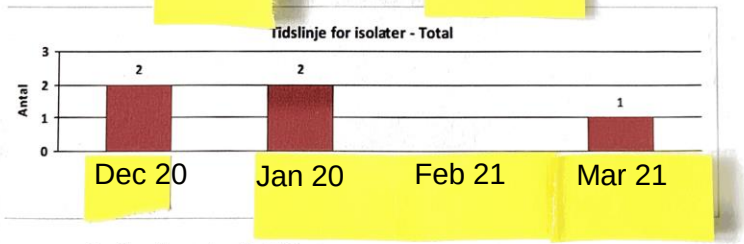
I alt 5 patienter, 4 mænd og 1 kvinde i alderen 48 til 77 år (gnm. alder 65,2 år).
Den første patient har rejseanamnese til

Figur 1 Alder og køn, KURS udbrudsdatabasen



Dato for første isolat

Dato for sidste isolat



a. Involverede sygehuse/institutioner

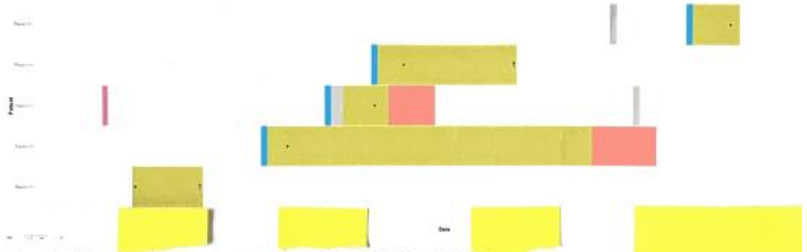
Patienterne har været indlagt på Intensiv Covid-afsnit Esbjerg. Patient 2, 3 og 4 har været indlagt samtidig.

Patient 1 og 5 har ifølge Landspatientregister data (LPR) ikke være indlagt samtidig med patient 2, 3 og 4, men været indlagt på samme stue (figur 2)

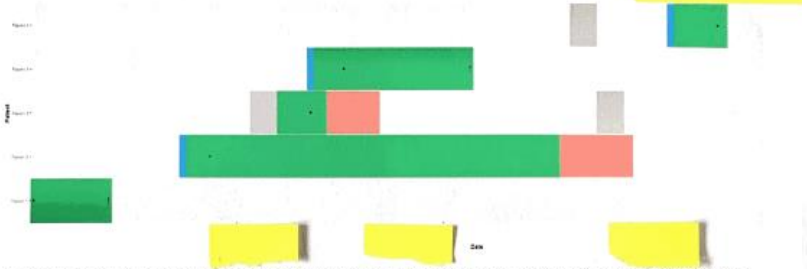
Tabel 1 Patienter med fælles indlæggelse og antal dage

Patient til	Patient	Afsnit	Dato ind	Dato ud	Antal dage
3	4	Sydvestjysk Sygehus, SVS E1 intensiv sengeafsnit (Esbjerg)			4
2	4	Sydvestjysk Sygehus, SVS E1 intensiv sengeafsnit (Esbjerg)			25
2	3	Sydvestjysk Sygehus, SVS E1 intensiv sengeafsnit (Esbjerg)			10

Figur 2 Indlæggelse på afdelingsniveau Anæstesiologisk afdeling. CoV Esbjerg



Figur 3 Indlæggelse på stueniveau, Anæstesiologisk afdeling. CoV, Esbjerg



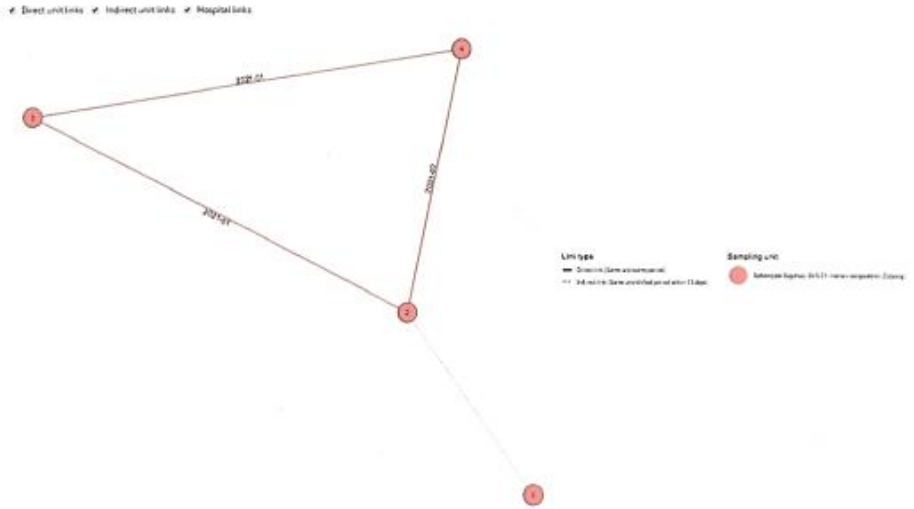
Figur 1 og 2: Blå farve er akutmodtagelse, Lyserød er SVS D1 sengeafsnit, mosgrøn/grøn er SVS E1 intensiv sengeafsnit. Grå farve er SVS D2 sengeafsnit.

Vi har fået særlige data på stueniveau fra Esbjerg, som er kørt i EpiLinx. Patient 2, 3 og 4 har flere fælles indlæggelser på pågældende stue.

Stuedata fra Esbjeg kørt i EpiLinx					
Patient til	Patient	Stue	Dato ind	Dato ud	Antal dage
2	4	Stuedata fra Esbjerg			15
2	4	Stuedata fra Esbjerg			4
3	4	Stuedata fra Esbjerg			1
2	4	Stuedata fra Esbjerg			5
3	4	Stuedata fra Esbjerg			4
2	4	Stuedata fra Esbjerg			4
2	3	Stuedata fra Esbjerg			10

Patient netværk

Figuren viser på baggrund af LPR-data direkte sammenfald i indlæggelse mellem patient 2,3 og 4. Patient nr. 1 har været indlagt under 14 dage før patient 2, men altså ikke samtidig.



Samlet vurdering

Ud fra indlæggelsesdata i LPR er der et klart sammenfald i indlæggelse mellem patient 2, 3 og 4 på stueniveau. Patient 1 og 5 har været indlagt på samme sengeafsnit. Vi har i tidligere udbrud set eksempler på spredning via miljø og kontaktsmitte, hvilket kan forklare spredning til patient 1 og 5.

Datcu R, Hasman H, Dyrgaard Rathmann A, Boczan, Bødker K, Østergaard Hansen F, Sanchez Garcia R, Song Z, Coia J.
Presented as a poster at the 2021 FIS meeting in Manchester UK

Region Syddanmark

Example of 6 month cgMLST analysis of all *C.difficile* isolates in South-Denmark region

Nedenfor er core-genome-MLST analyse på C . difficile for 1. halvår 2022 (både sentinel og løbende indsendelse). Træet indeholder kun isolater med vellykket wgs (core-genom%>95).

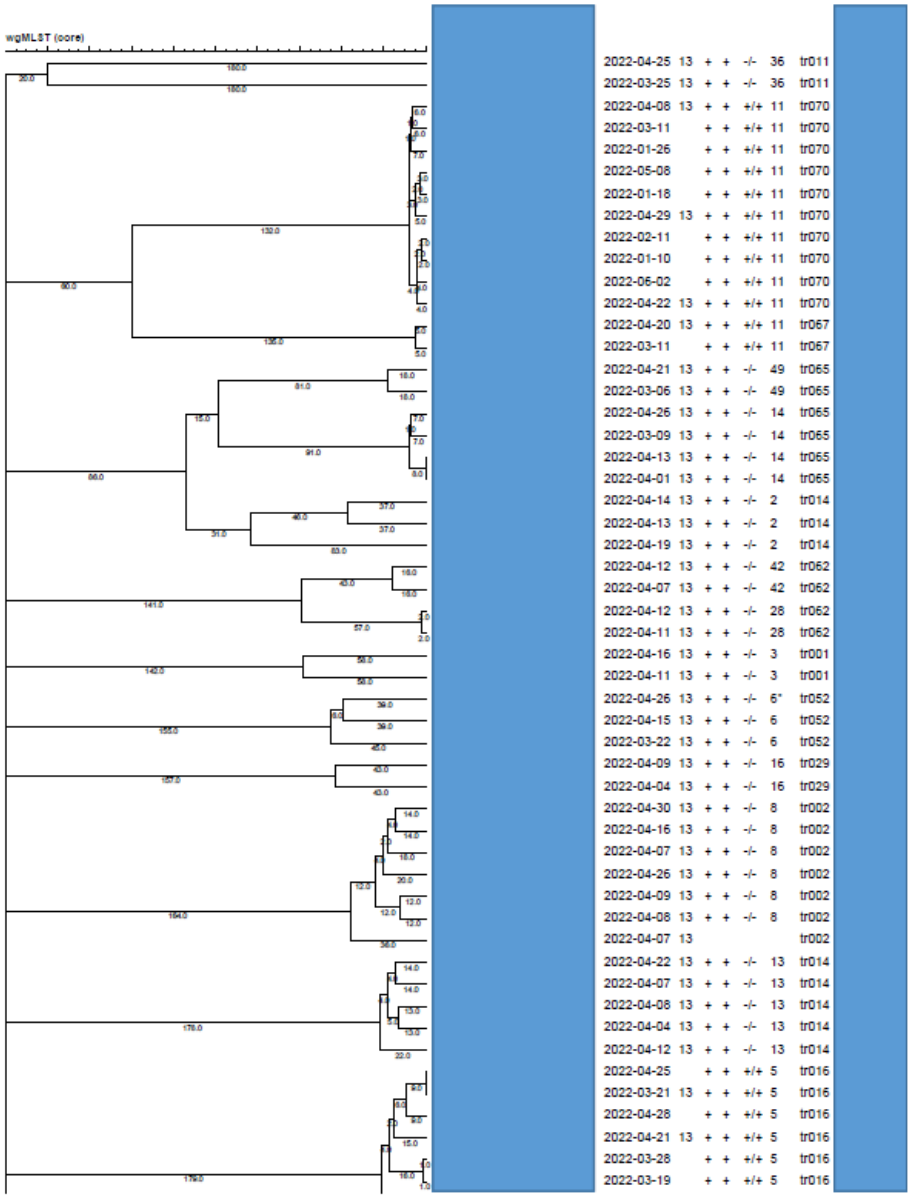
Bemærkninger til træet:

cgMLST-træ baseret på ca. 2000 alleler (BioNumerics).

Allel-forskelle angivet på målestok, lange grene er dog udenfor målestok, dvs. nogle overskrider 200.

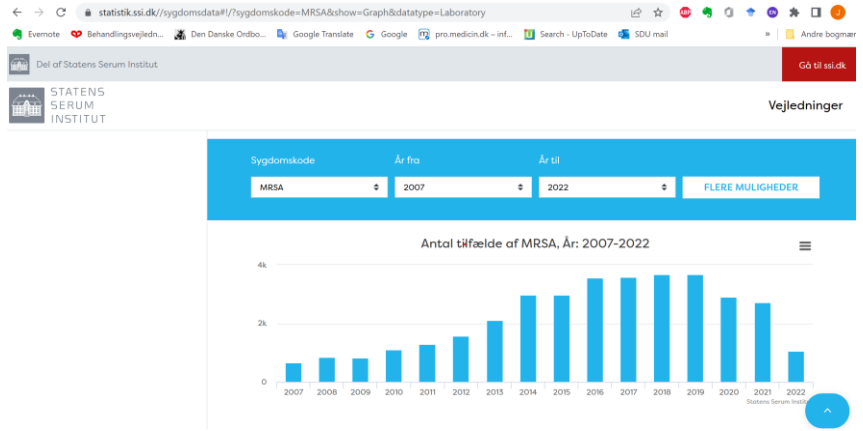
Kolonne-forklaring til træet:

1) SSI prøve-nr.
2) CPR-nr
3) KMA-prøvedato (år-mdr-dag)
4) Sentinel periode 13=forår 2022, tomt=løbende indsendelse
5) tcdA
6) tcdB
7) cdtA_cdtB
8) MLST type
9) TRST type
10) KMA



Advice and information from the NRL

- Investigation of the individual patient
- Investigation of outbreaks
- Troubleshooting of existing methods
- Implementation of new methods
- Meetings
- Epidemiology
  - Local/Regional
  - National
  - International
- Communication
  - Reports
  - Online
  - Publications



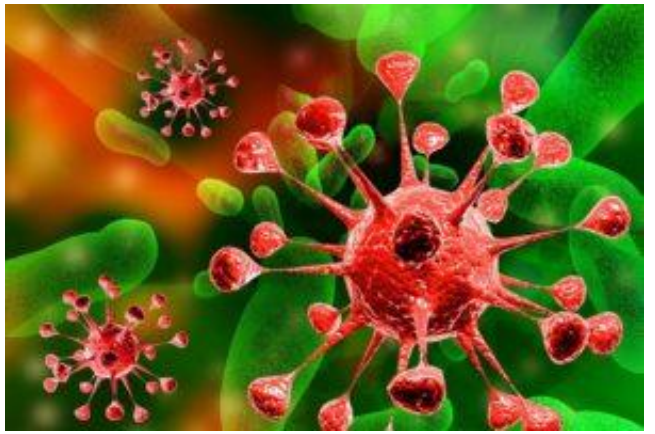
Region Syddanmark

Other uses of WGS from the perspective of the clinical laboratory

Region Syddanmark

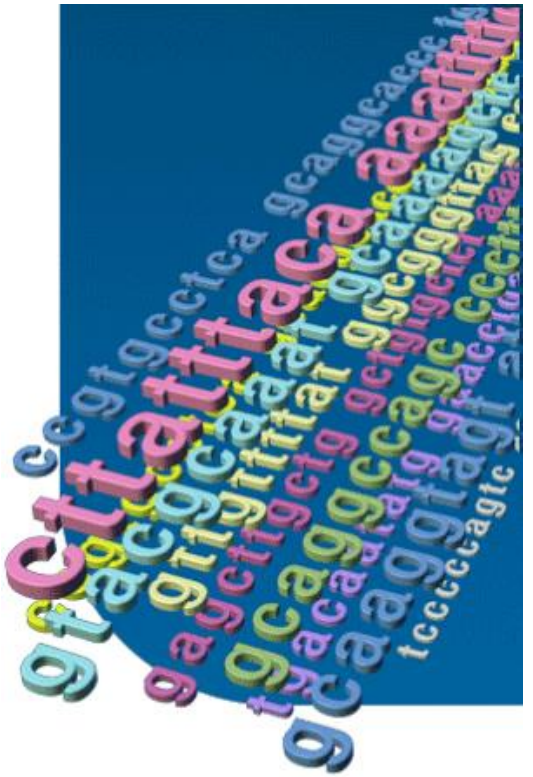
WGS will be applied to an ever-increasing range of pathogens

- **Bacteria**
  - *Mycobacterium spp*., *Listeria spp.,* MDROs, pneumococci, meningococci, *Legionella spp.*, coagulase-negative staphylococci
- **Viruses**
  - SARS-Cov2, SARS, MERS, HIV, Hepatitis (HAV, HBV, HCV, HEV), Influenza, Monkeypox
- **Fungi**
  - *Candida* spp., *Aspergillus* spp.
- **Parasites**
  - *Plasmodium* spp., *Schistosoma* spp., *Cryptosporidium* spp.



WGS and diagnostics

- Metagenomic analysis
  - Sterile body fluids
    - CSF, joint aspirates
  - Typing from sample
  - Microbiome and disease
    - Dysbiosis
    - Modulation of other disease states
  - Diagnostic microbiology
    - ID, resistance determination and epidemiology data



Current developments

Danish COVID-19 genomics consortium

- To assist public health authorities to monitor the spread of SARS-CoV-2
- Large-scale SARS-CoV-2 sequencing capacity was initially established at Aalborg University and local sequencing capacity at Statens Serum Institute and Hvidovre Hospital
- Since then expanded to include almost all KMAs in Denmark (Illumina/ONT)

https://www.covid19genomics.dk/about

Listeria outbreak in Denmark

- Current outbreak with ST 8 and ST11
- Majority of sequencing performed centrally at SSI
- Local sequencing (ONT) at KMA Slagelse

The future?

Longer term (>5 years?)

- Microbiology will change
  - WGS will move into the diagnostic laboratory
  - Decreasing cost and increasing automation
  - Automated analysis
  - Reliably predict AMR
  - Sequence direct from specimen
  - ID, AMR, virulence, Typing data all at the same time (plus more)
- Very important tool in tackling AMR problem
- Need for more epidemiological and IPC skills
  - Decline in number of “sporadic” cases
- IPC practices will be revisited and refined based on an expanded knowledge base facilitated by WGS

What matters for the clinical laboratory?

- **Quality**
  - Accuracy
  - Turnaround time
  - Reliability
  - Responsiveness
  - Availability
  - Requesting and reporting
  - Feedback
  - User involvement
- **Improved outcome**
  - Improved patient outcome
  - Outbreaks controlled
  - Infections prevented
  - Emergence of resistance reduced

Region Syddanmark