

Clostridioides difficile

Setting up national surveillance in Denmark

OUTLINE:

- Danish healthcare organization
- Introduction to *C. difficile*
- Sentinel surveillance: design/collaboration/outcome/challenges

Hospitals/DCM ↔ NRL/SSI

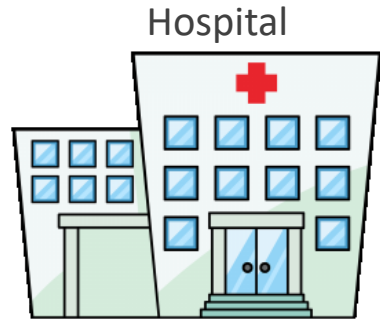
- Conclusion

Relative new pathogen: story of starting from zero

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Healthcare Organization

Focus: gastrointestinal bacterial infections



Hospital

Dept. Clinical Microbiology (DCM)

DCM: Gastrointestinal infections (GI)



DANISH MINISTRY OF HEALTH



DANISH HEALTH AUTHORITY

Data, samples info, diagnostics, typing, epidemiology, education

Annual meet. (2-day)
Numerous study groups:
ex. virus, parasites, sepsis, AMR, GI ...

Biannual board meet.
Annual GI meet (1-day)
Ad hoc contact



Ministry of Food, Agriculture and Fisheries of Denmark



Danish Veterinary and Food Administration

Statens Serum Institut (Public Health)

Infectious diseases
National surveillance
Epidemiology
National reference lab
Rare diagnostics

Dept. Bacteria, Parasites & Fungi

Unit Food borne Infections

Central outbreak group
Weekly meeting
Zoonotic outbreaks

Dept. Epidemiology

DTU Food

Veterinary and Food Administration



The story of *C. difficile*



Relative new pathogen

1935 - *C. difficile* isolated

1978 - AB associated diarrhea
- Clindamycin associated diarrhea

2000 - Worldwide hospital outbreak of ST1/027 (FQ-resistant)
(USA → Europe, Australia (Asia))
- Major cause of hospital/AB associated diarrhea

2010 - Worldwide continued nosocomial transmission/outbreak
- Worldwide increase in community associated infections (many non-ST1 types)

timeline

C. difficile - introduction



Gram+, anaerobic, rod

Oral ingestion (fecal/environment)

Spore-forming: resistant to O₂ and many disinfectants, dormant

>120.000 *C. difficile* infections (CDI)/year in EU

Recurrence 15 - 30%

Case fatality rate ca. 15%

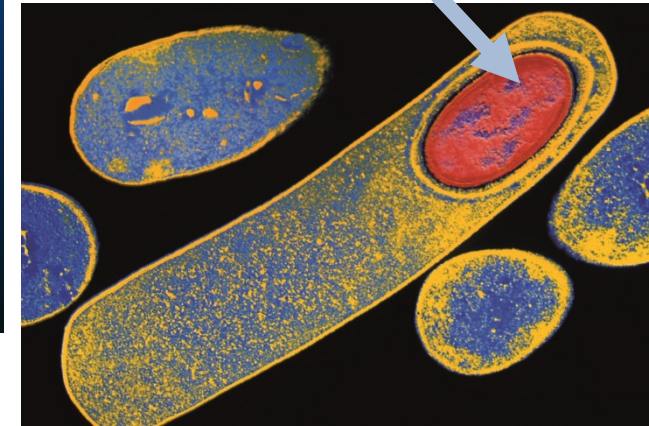
Pseudomembranous and fulminant colitis, toxic megacolon, sepsis

Costs 6.3 billion per year in US

Vegetative cells



Endospore



Pseudomembranous colitis

C. difficile



Toxins: TcdA, TcdB & binary toxin (CdtAB)

Antibiotic associated diarrhea (1978)

Hospital associated diarrhea

70s: clindamycin, 80-90s: cephalosporins, 00s: fluoroquinolone

Increasingly community acquired (One Health)

Antibiotic risk: broad spectrum:

Carbapenems

Cephalosporins

Fluoroquinolone

Clindamycin

Antibiotic treatment: VANCO, FIDAX, (METRO ↘)

Fecal transplantation, monoclonal AB (expensive)

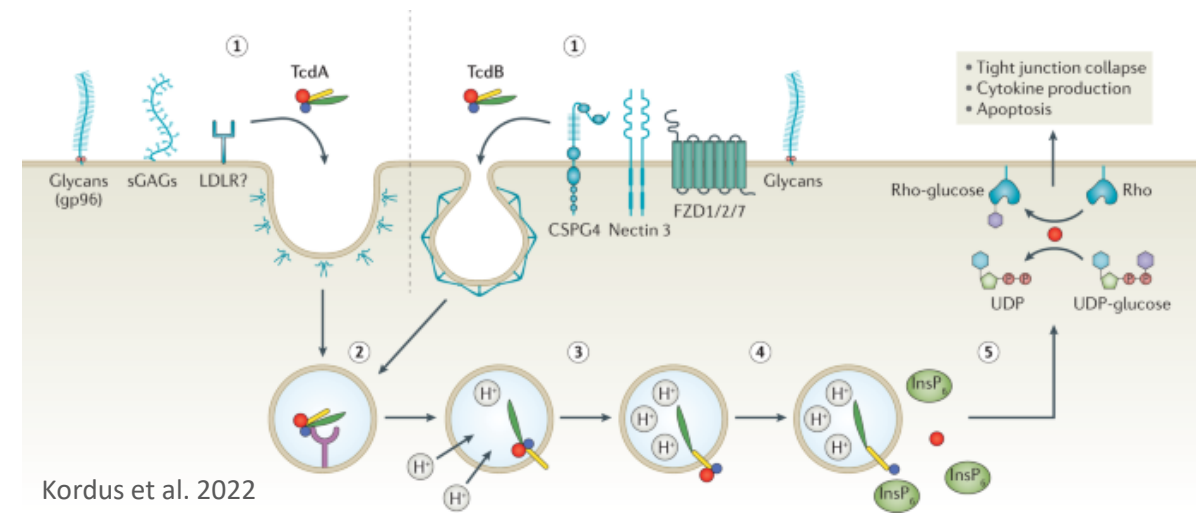
Major ST epi clones (PCR-ribotype):

ST1(027): nosocomial/MDR/hypervirulent ↘

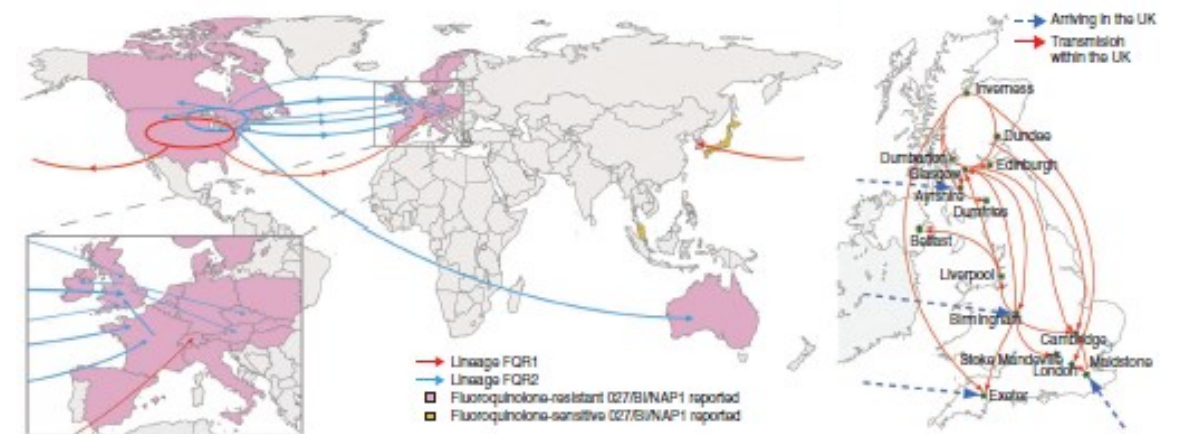
ST11(078): veterinary/MDR/hypervirulent ↗

ST2(014/020): community ↗

Main toxins: TcdA and TcdB



Global spread of ST1(027) early 2000s



He et al. 2013

National *C. difficile* surveillance in Dk

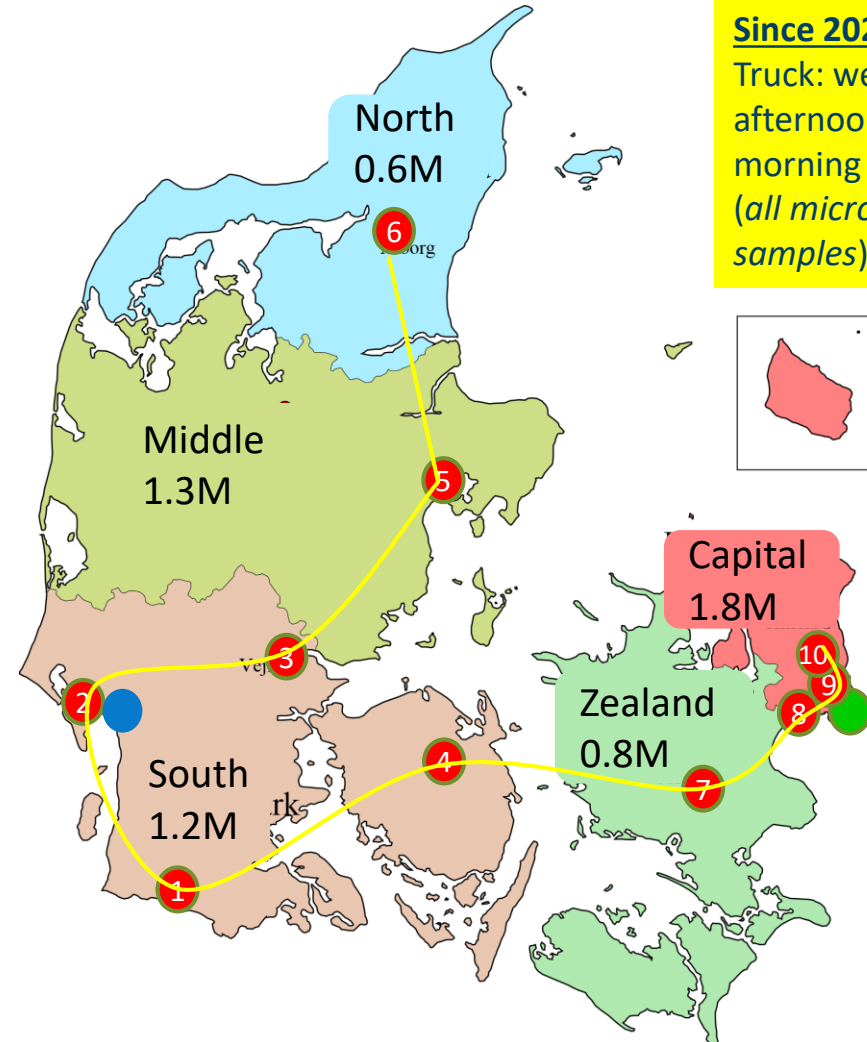


Denmark 5.6M, 5 healthcare regions

- 10 Dept. Clinc. Microbiol. (DCM)
- Copenhagen, SSI: National Ref. Center

Since 2020

Truck: weekday
afternoon, at SSI next
morning
(all microbiological
samples)



- 2000** - *C. difficile* ST1/027 USA → EU
- 2006** - First cases of ST1/CD027 in Dk ●
2006: PCR ribotyping/toxin PCR
- 2008** - Dk national surveillance (*all year*)
Danish Health Authority (mandatory):
1) Severe clinical outcome
2) Outbreak
3) Binary toxin positive
4) FQ resistant/ST1/027
2013: Tandem repeat seq. typing/toxin PCR
- 2016** - *Sentinel Surveillance* (optional)
All 10 DCM's, all toxigenic
1 month spring + fall
2018: WGS/BioNumerics/AMRFinder
- 2023** - *All year*: optional
- *Sentinel*: mandatory/notifiable disease

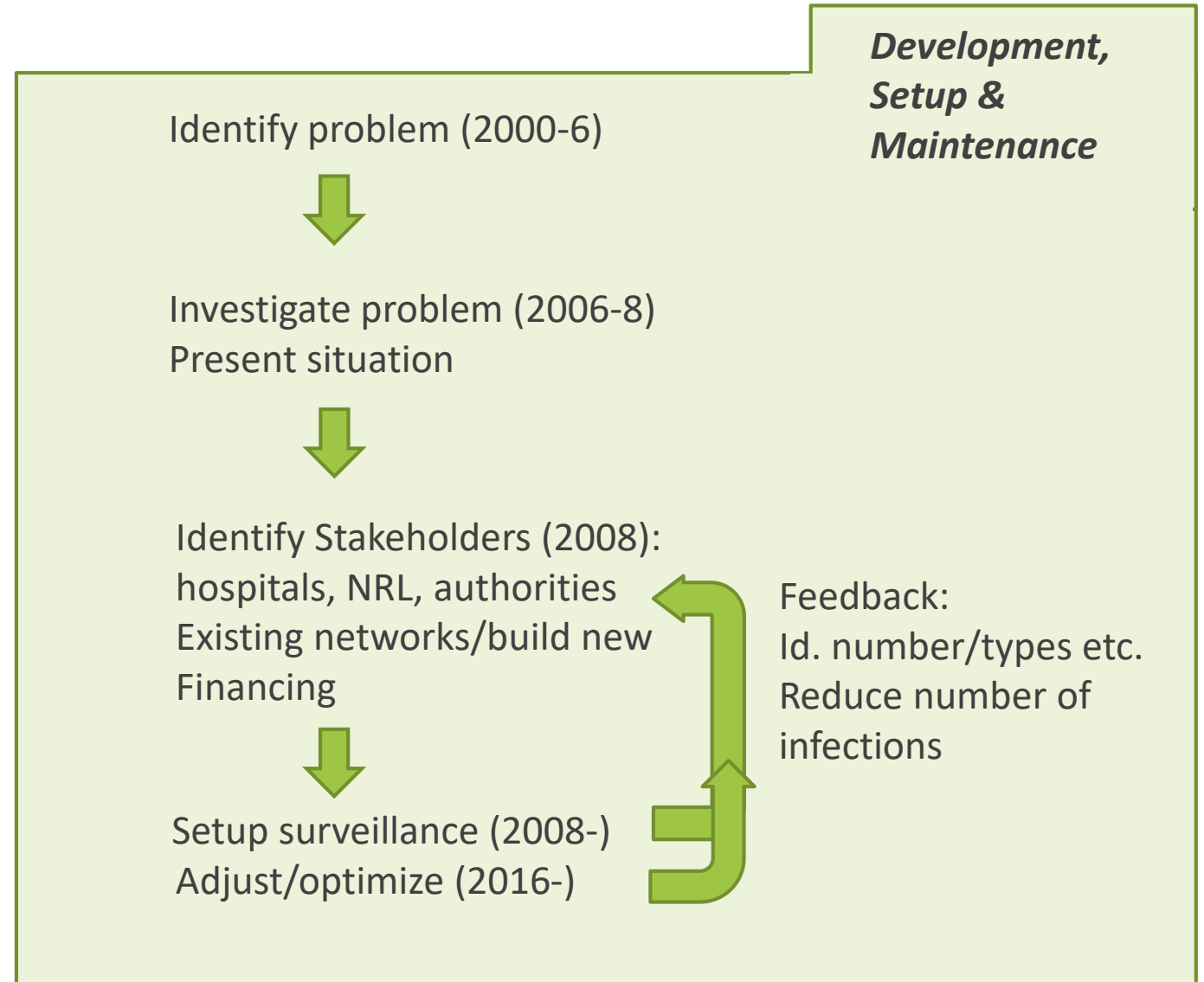
timeline

National *C. difficile* surveillance in Dk

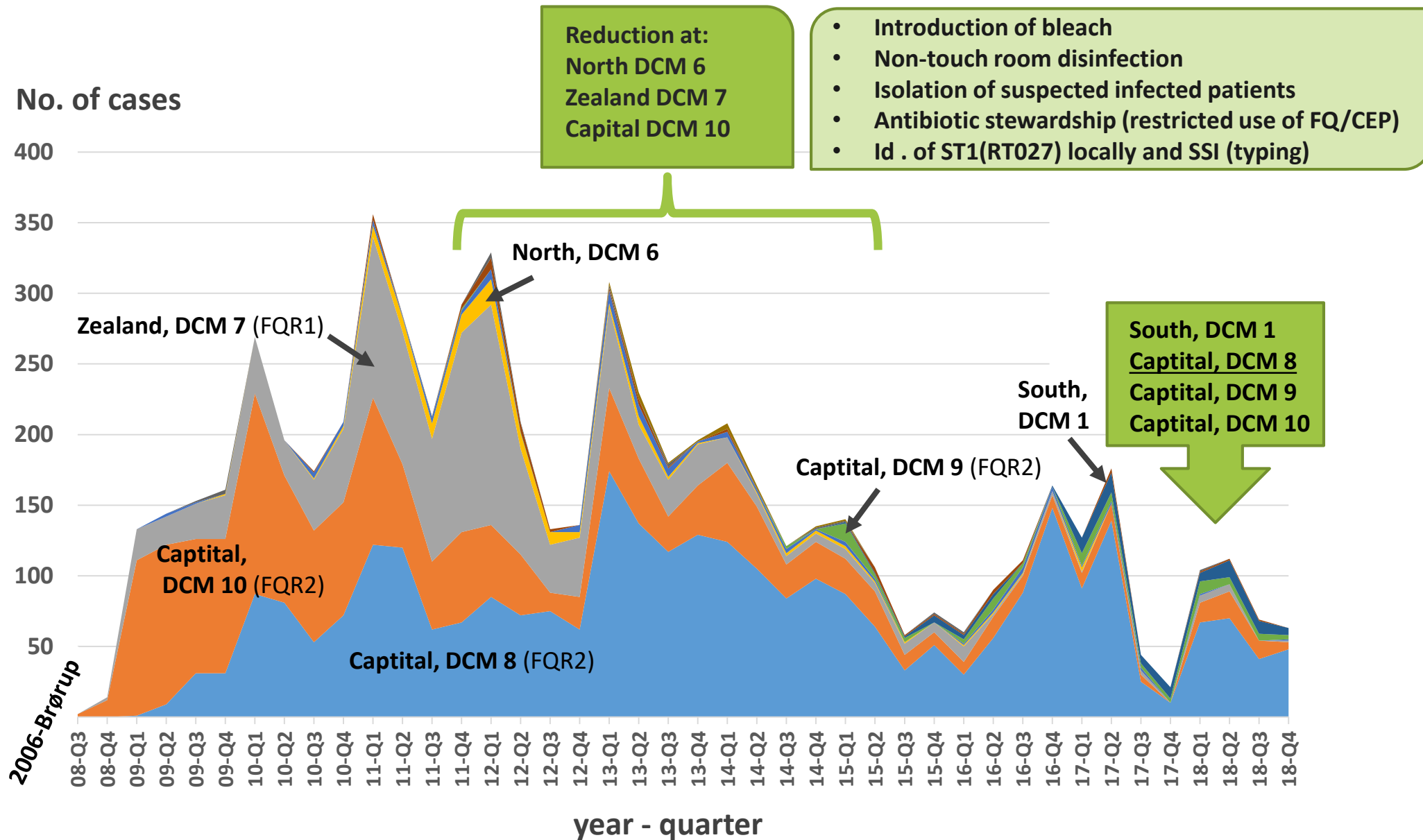


- 2000** - *C. difficile* ST1/027 USA → EU
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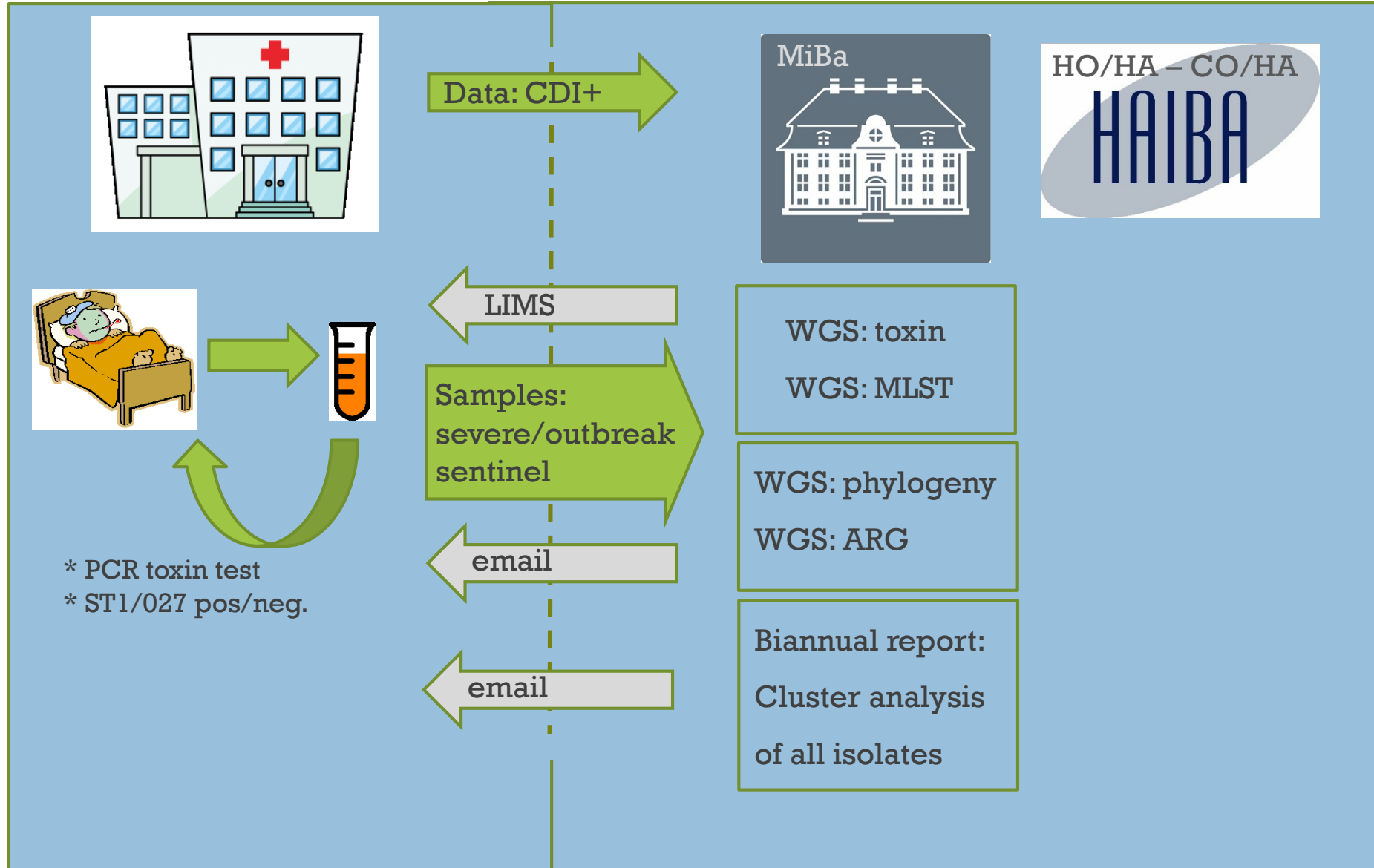
timeline



ST1/027 per quarter/DCM, 2008-18



Sample/data-flow: hospitals (DCMs) ↔ SSI



C. difficile WGS pipeline

C. difficile specific

Sample

Culture

10 min 62C in PBS,
selective plate
(ChromID)
→ Blood plate

2-step lysis:
Lysozyme: 37C
Proteinase K: 56C

Core Sequencing Unit (all species)

DNA extract
MagnaPure

Library prep.
Nextera XT, Hamilton

Sequencing
NextSeq

QC

Cdiff 0,5 ng/μl, others 0,3 ng/μl

Toxins:

- *tcdA*
- *tcdB*
- *cdtA/B*
- *tcdC*: 0,18, 39 og 54, Δ117

Typing:

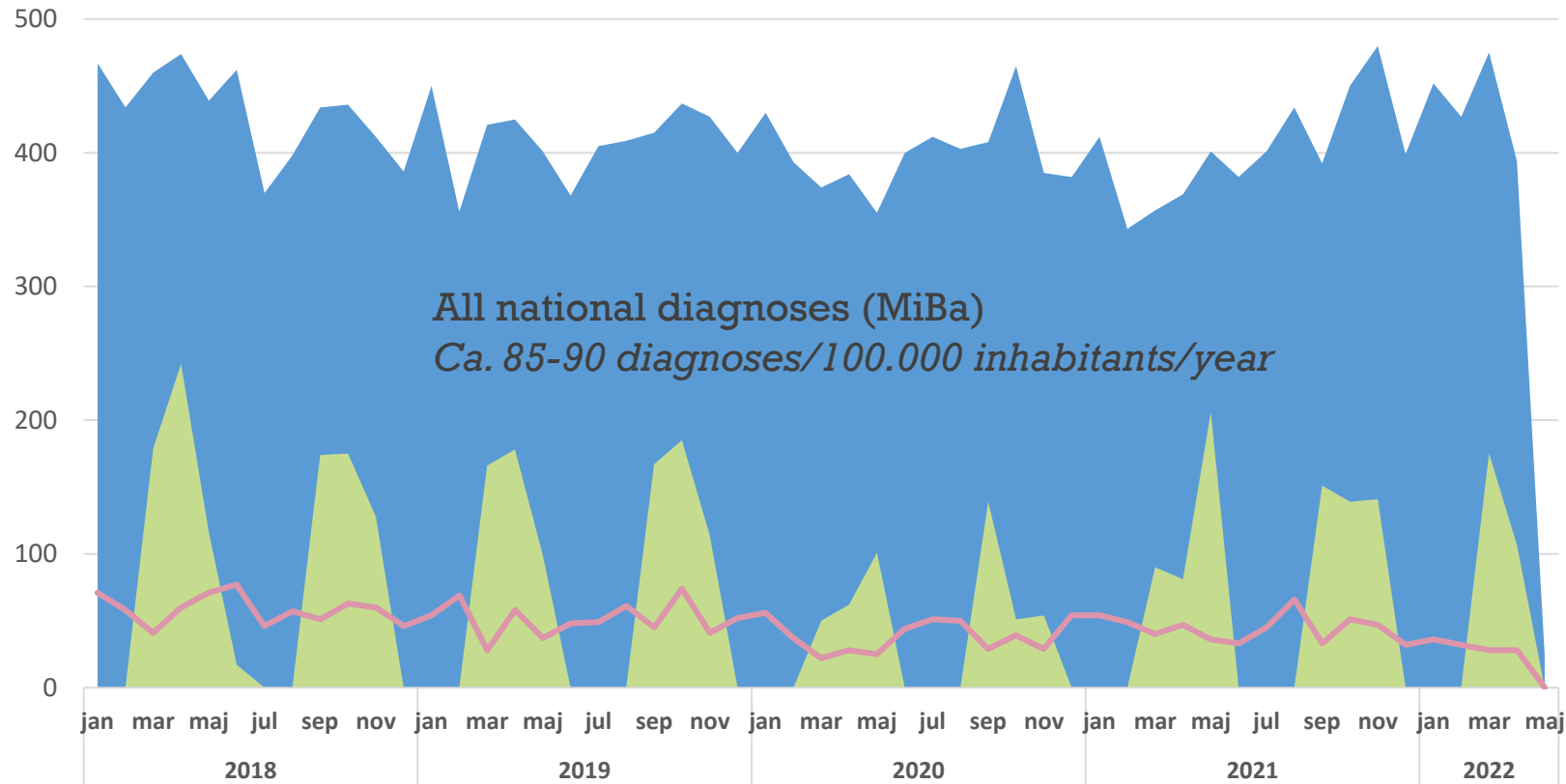
- 7-loci MLST
- cgMLST / wgMLST / SNP
- ARG (AMRFinder)

Sequence analysis



Dk national surveillance

Number



WGS typing at SSI

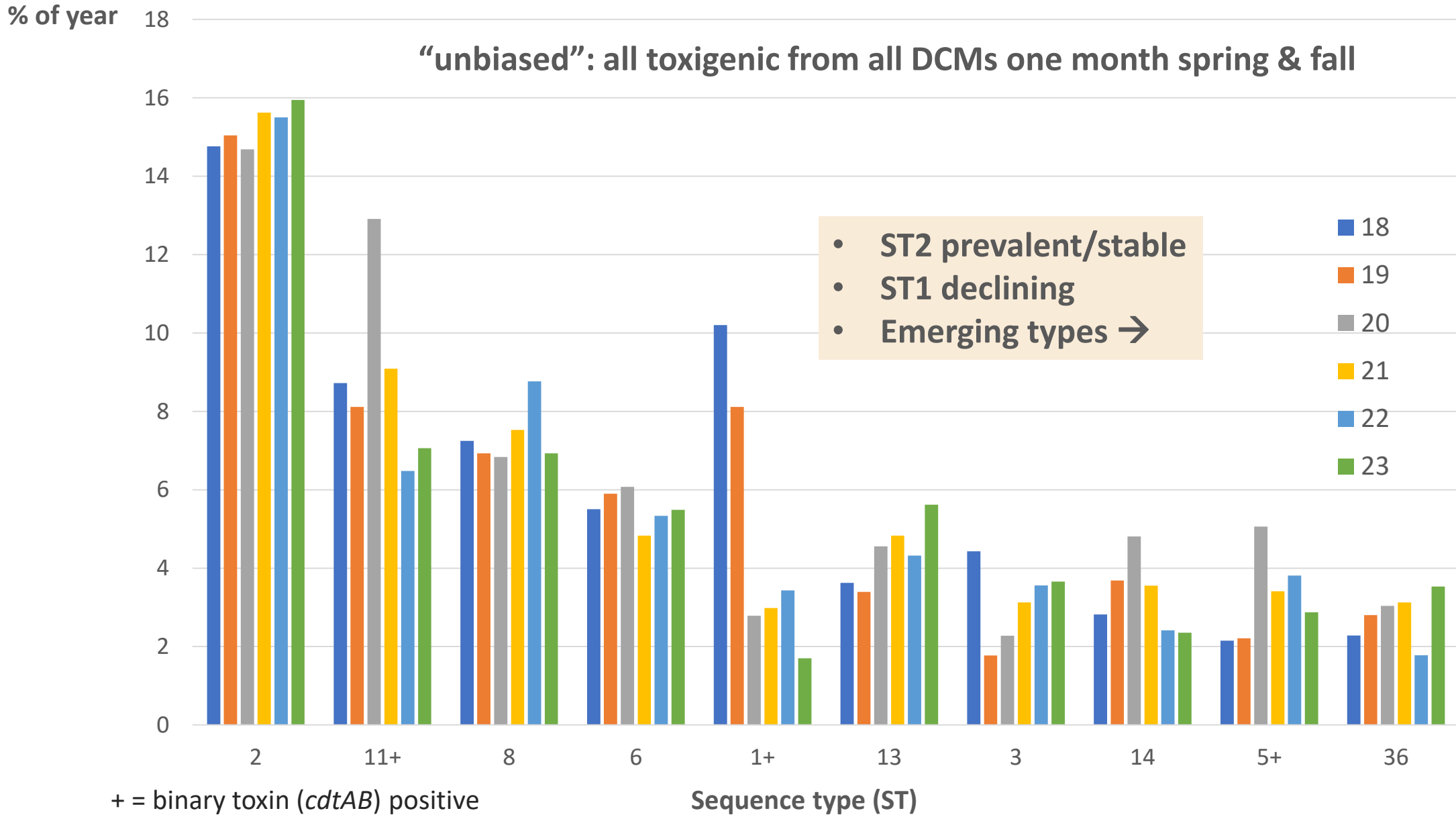
- All year since 2008 (ca 50/month)
 - 1) Severe clinical outcome
 - 2) Outbreak
 - 3) (Binary toxin positive)
 - 4) (ST1/027)

- Sentinel since 2016 (ca 800-1000/yr)
All 10 DCM's, all toxigenic
1 month spring + fall



Ca. 25% of all national diagnoses

Dk clinical sentinel surveillance 2018-23

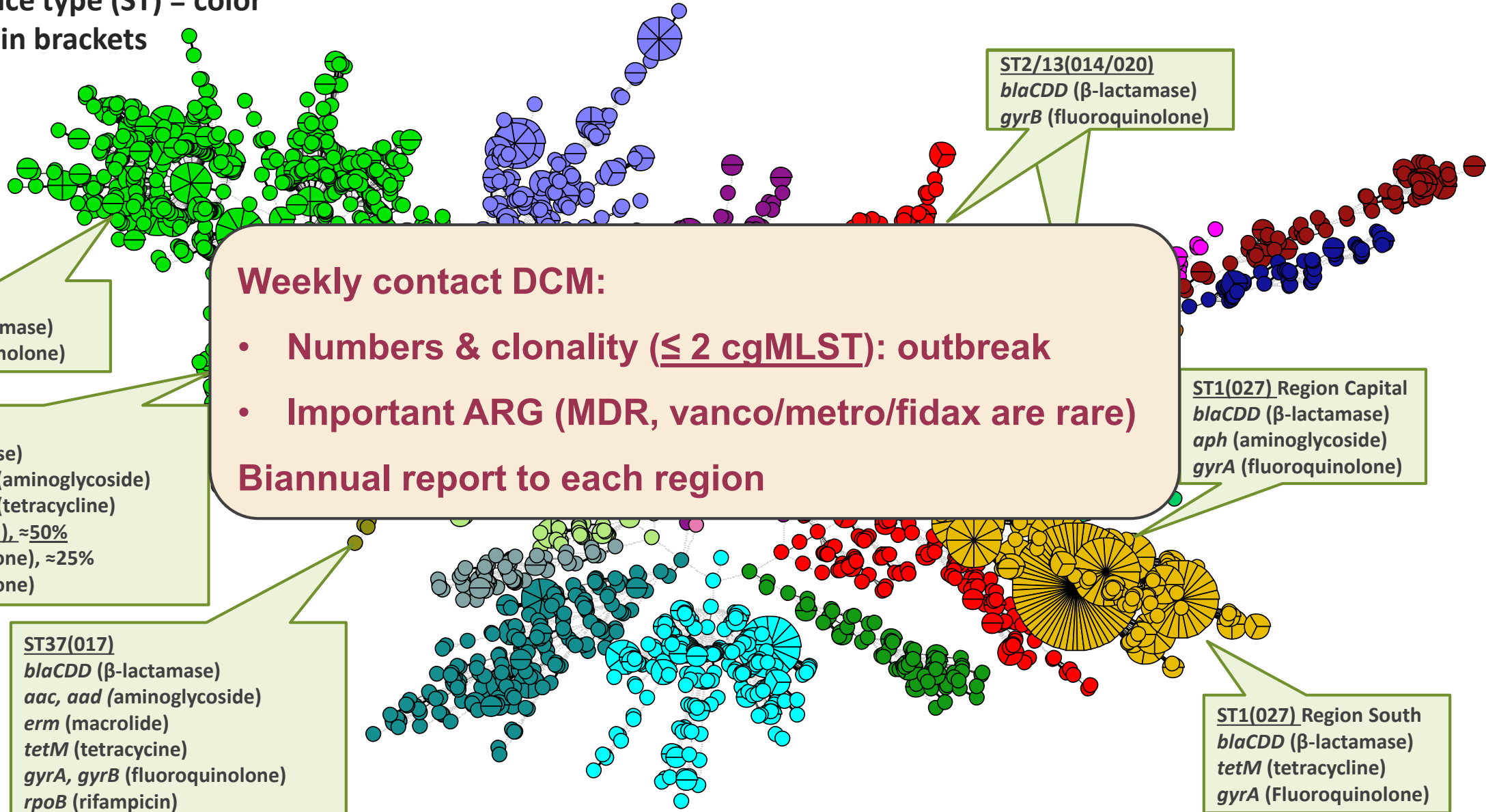


C. difficile population structure in Dk

MST cgMLST (similarity \approx outbreak/transmission)

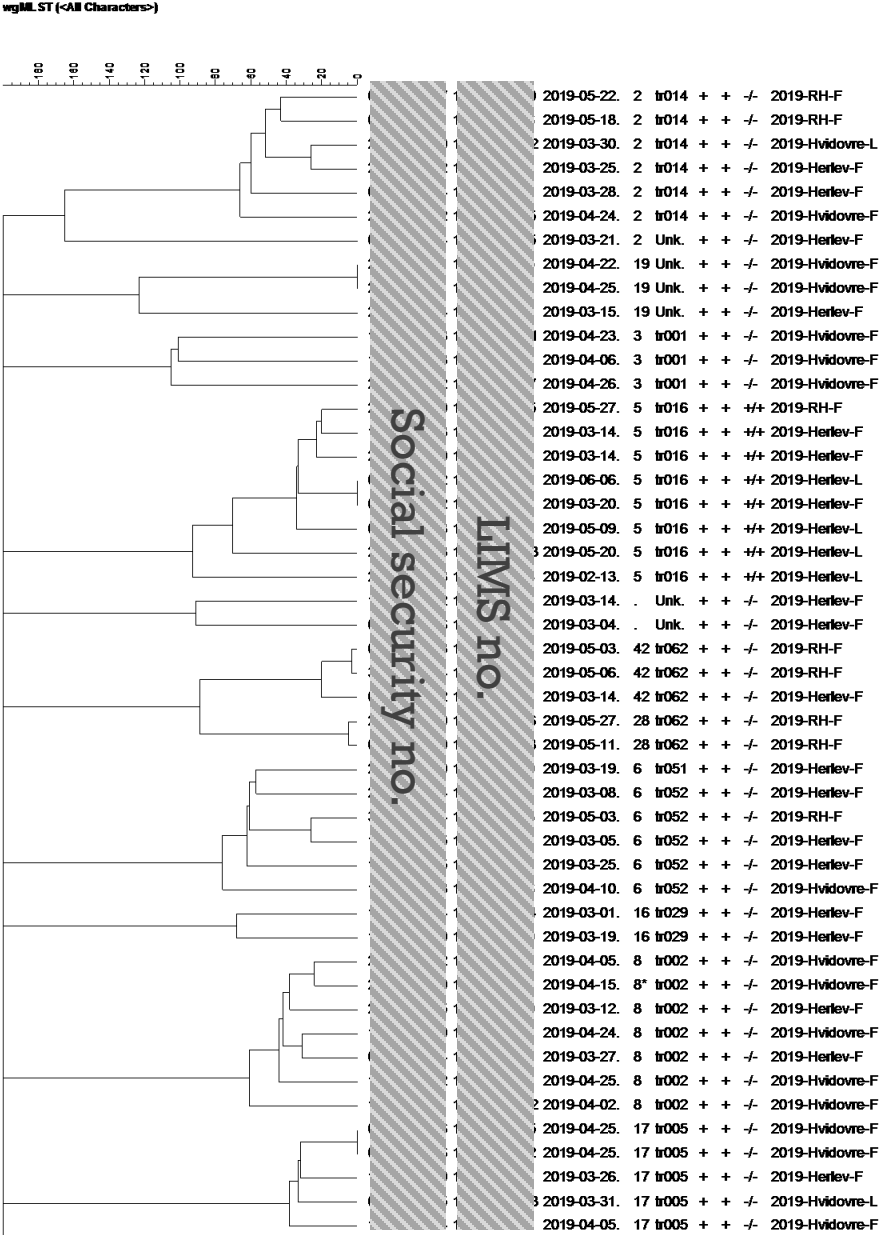
Major sequence type (ST) = color

PCR ribotype in brackets



Biannual data back to DCMs (5 regions)

ST	DCM-1	DCM-2	CDM-3	Total
No growth	15	30	3	48
11	27	1	6	34
1	4	22	5	31
103	10	4	1	15
2	6	6	3	15
5	10	1	1	12
6	5	1	1	7
8	3	4		7
17	2	4		6
14	2		2	4
3		4		4
-	3			3
19	1	2		3
42	1		2	3
13	1	2		3
37		3		3
36	1	1		2
28			2	2
16	2			2
34	2			2
512	2			2
35		2		2
26	1	1		2
431*			1	1
223	1			1
133		1		1
54			1	1
44	1			1
55		1		1
129		1		1
58	1			1
Total	105	96	29	230



Sentinel surveillance in summery

NRL-DCM collaboration:

- The more (samples & DCMs) the merrier (representativeness versus economy)
- Mandatory (Health authorities) versus optional & sample type
- Local outcome: id. ST, transmission/outbreak, hotspots, and ARG
- National outcome: types/time/geographical changes
- International outcome: compare to and support international surveillance

Sentinel with all toxigenic (pros/cons)

- + “unbiased” i.e. all, regardless of binary toxin, severity, outbreak, etc. + emerging
- + Temporal and geographical comparison of STs and ARG
- ÷ Less observations between sampling periods: transmissions/outbreaks
- ÷ Still expensive to do WGS on a subset (e.g. 15%) of all national isolates

Challenges

Denmark: no uniform case definitions and different diagnostic methods (≠ ECDC guidelines*)

- difficult to compare intra- and international and to know exact numbers

Direct PCR diagnostics – limited culturing – develop metagenomics at NRL/SSI

Who is paying? Ex. hospitals change from culture to PCR => expensive culture at NRL/SSI

*) <https://www.ecdc.europa.eu/en/clostridioides-difficile-infections>

Sentinel surveillance in summery

How it got started

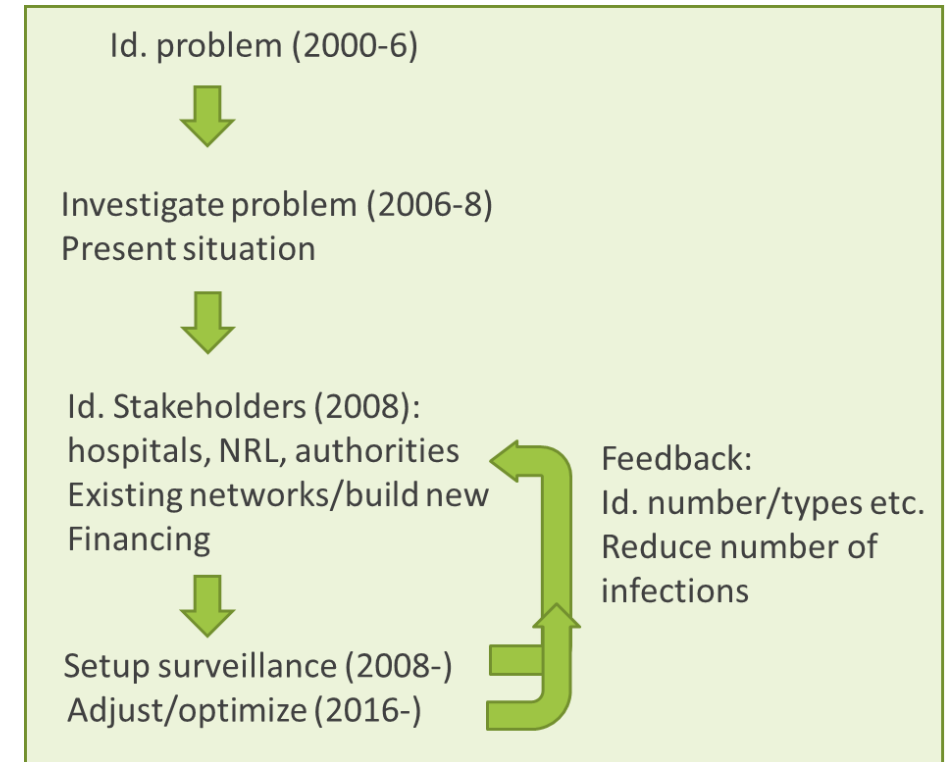
- Identification of problem: increase of international/hospital acquired *C. difficile* (ST1/027)
- Setting up small screening analysis at SSI
- Existing network between DCMs and SSI
- Presenting overview of ST1 (and others) in Danish hospitals
- Mandatory screening program: Danish health authorities, DCMs and SSI

How its maintained

- Weekly results back to hospitals; id. of outbreaks, hypervirulent clones and ARG (→hygiene/patient isolation)
- Biannual report for entire hospitals/healthcare region; retrospective analyses (→ex hospital hotspots, patient transmission)
- Common publications, meetings/collaborations
- Mandatory: *all-year*: 2008-23, *Sentinel*: 2023->
- Financial prioritization (state grant “antimicrobial resistance” 2018->)

How is the future

- Hospitalized patients are continuously prone to infections/*C. difficile*
- Antimicrobial resistance continued importance
- Community acquired is increasing: other programs?
- One Health: identify community sources for reduced spread



Sentinel surveillance in summery



SURVEILLANCE

Sentinel surveillance and epidemiology of *Clostridioides difficile* in Denmark, 2016 to 2019

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from each DCM/
hospital

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Background: Since 2008, Danish national surveillance of *Clostridioides difficile* has focused on binary toxin-positive strains in order to monitor epidemic types such as PCR ribotype (RT) 027 and 078. Additional surveillance is needed to provide a more unbiased representation of all strains from the clinical reservoir. **Aim:** Setting up a new sentinel surveillance scheme for an

changes in *C. difficile* infections in Denmark, including emerging types, regardless of binary toxin status.

Introduction

Clostridioides difficile infection (CDI) is the leading cause of antibiotic-associated diarrhoea and a common nosocomial pathogen, often leading to severe