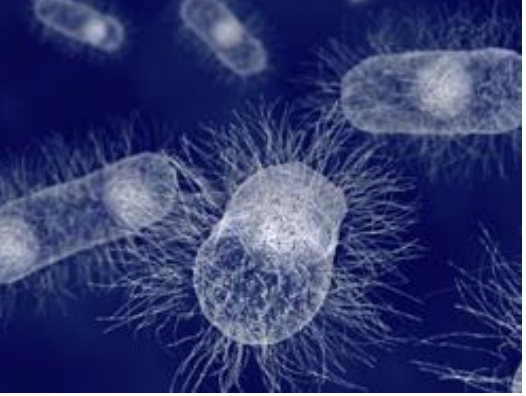
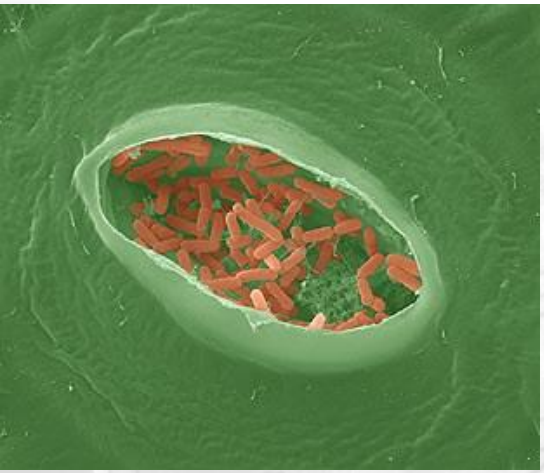
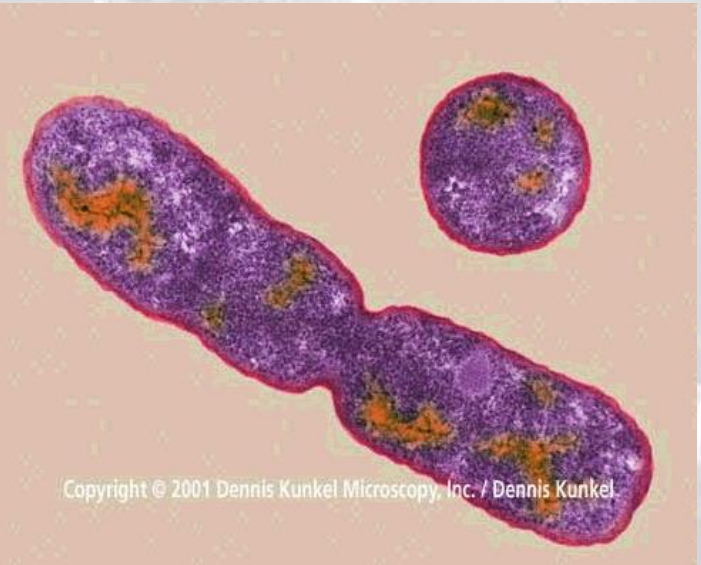






STATENS SERUM INSTITUT

VTEC – HUSEC Helgenomsekvensering (WGS) erfaringer 2015-16

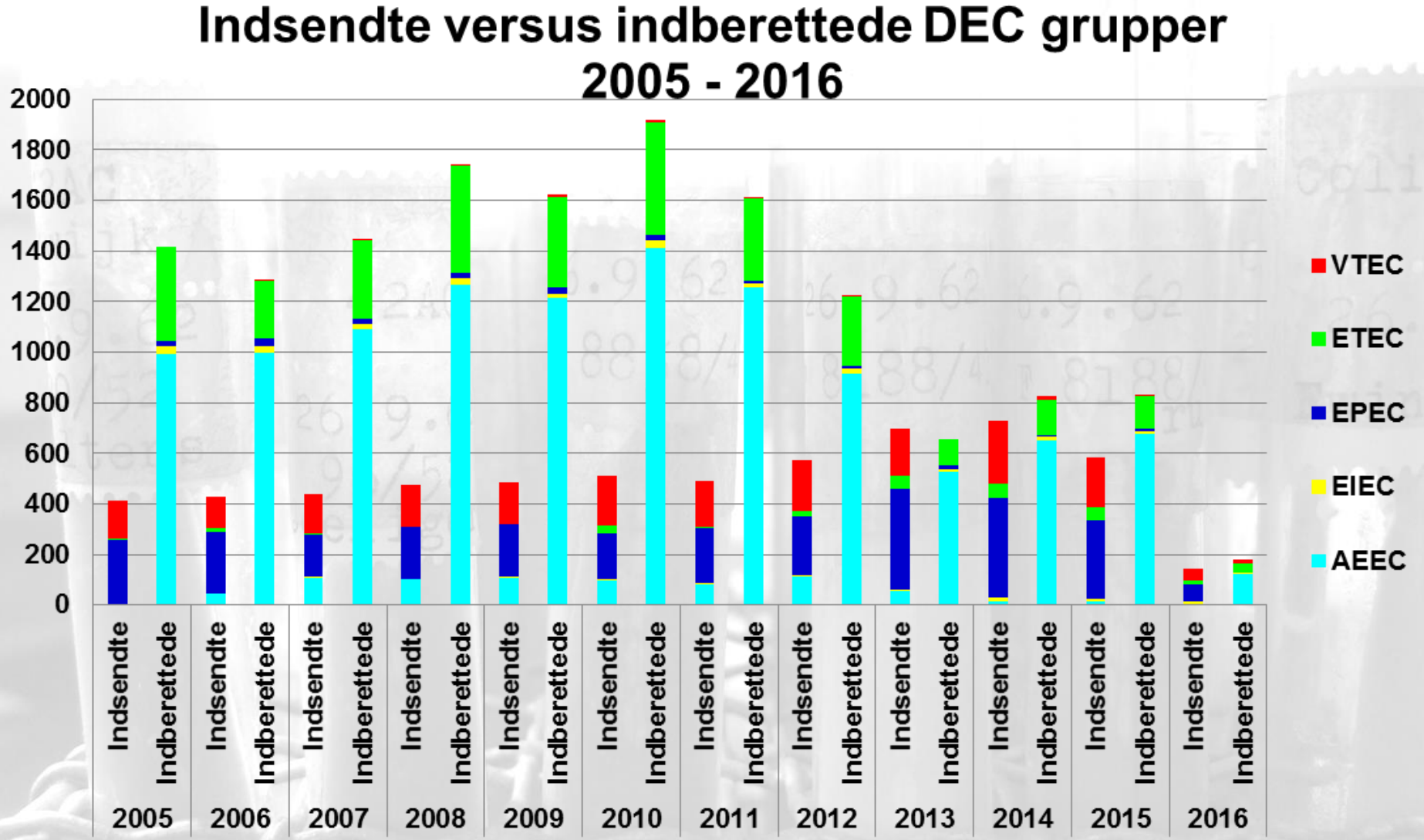
Flemming Scheutz

WHO Collaborating Centre for Reference and Research on *Escherichia* and *Klebsiella*

Fødevarebårne infektioner

Mikrobiologi & Infektionskontrol
STATENS SERUM INSTITUT

DEC i Danmark 2005 - 2016



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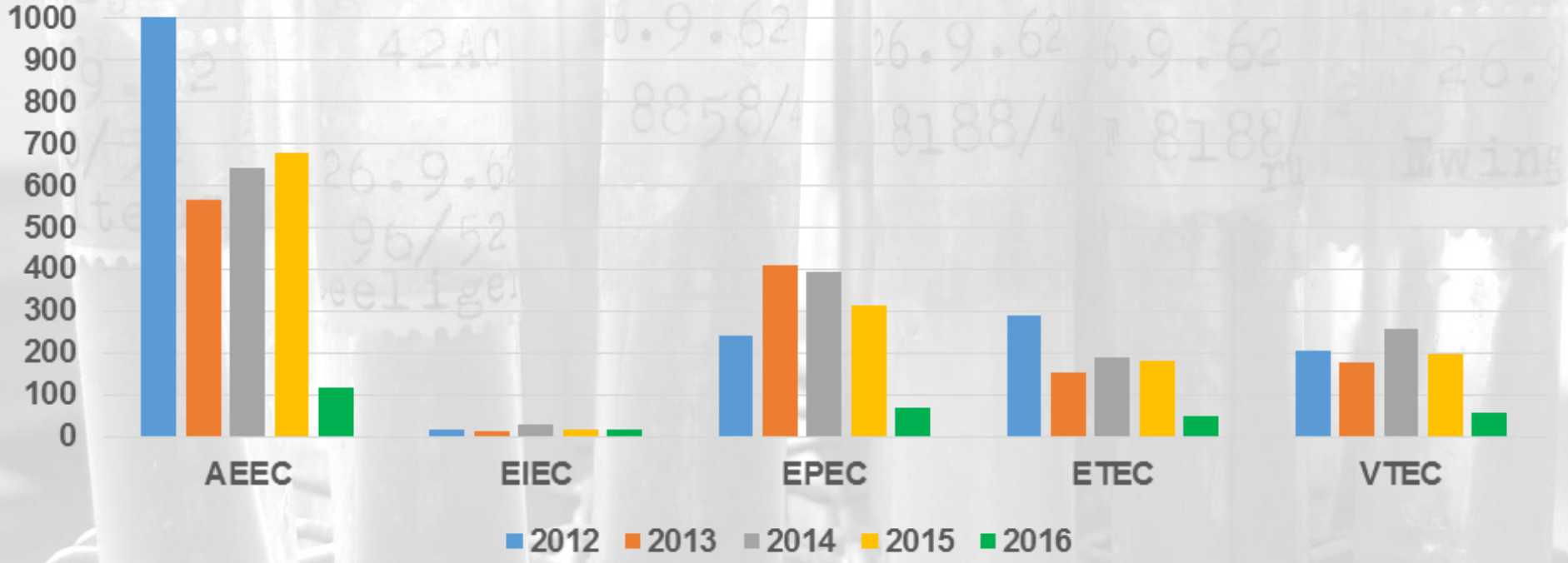
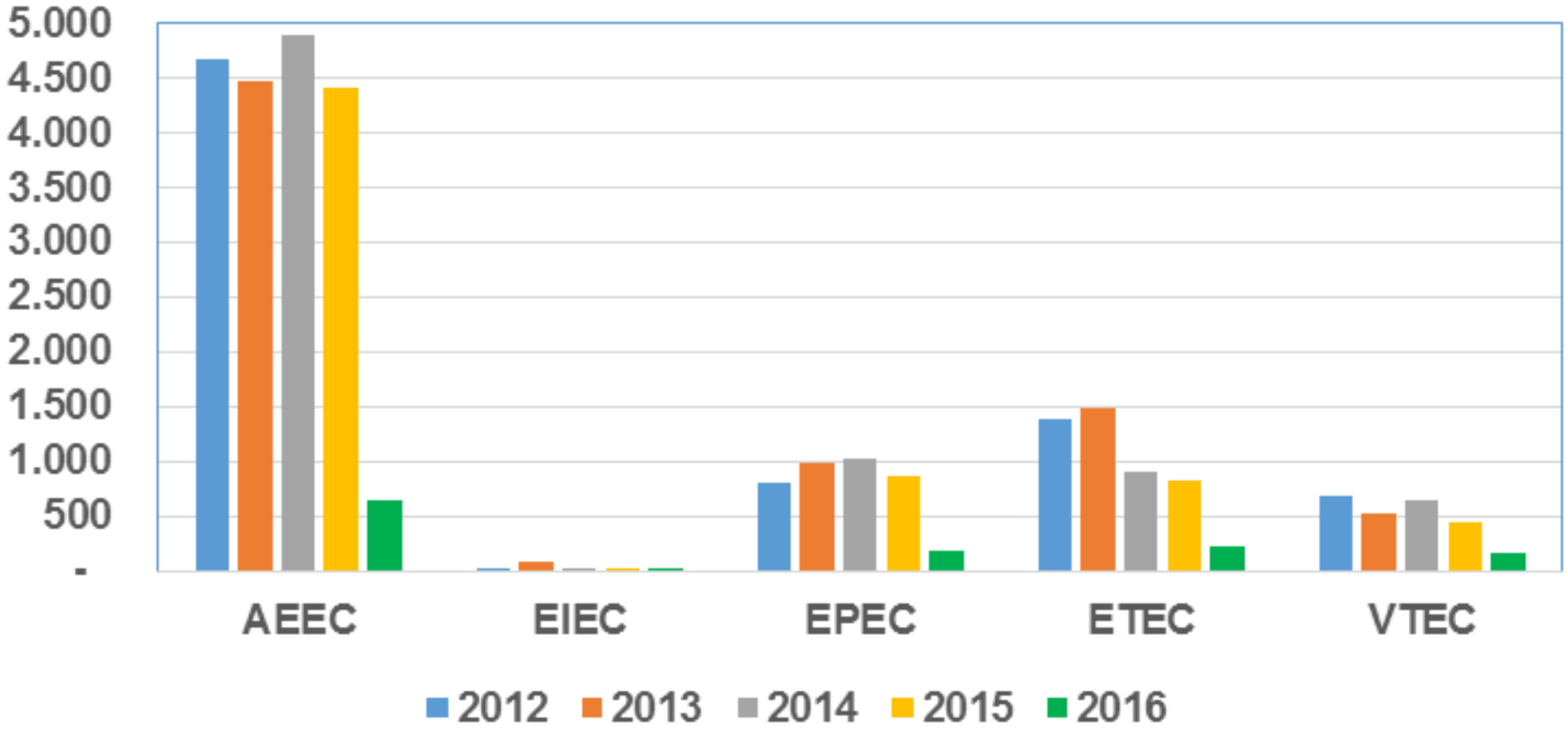
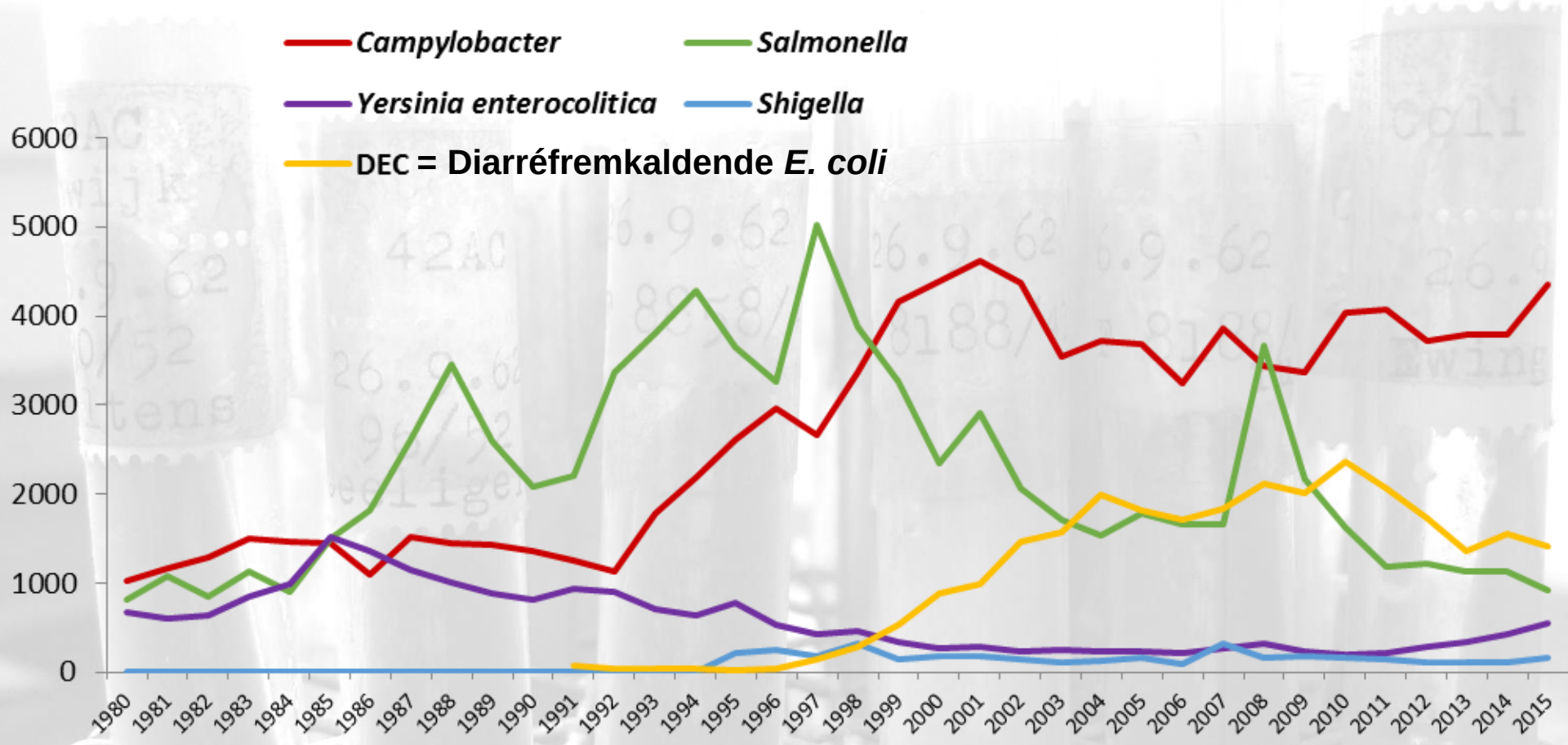
Registrerede antal tilfælde af DEC 2012-1015



Muligt antal tilfælde af DEC; 2012-2015
Ekstrapolerede tal

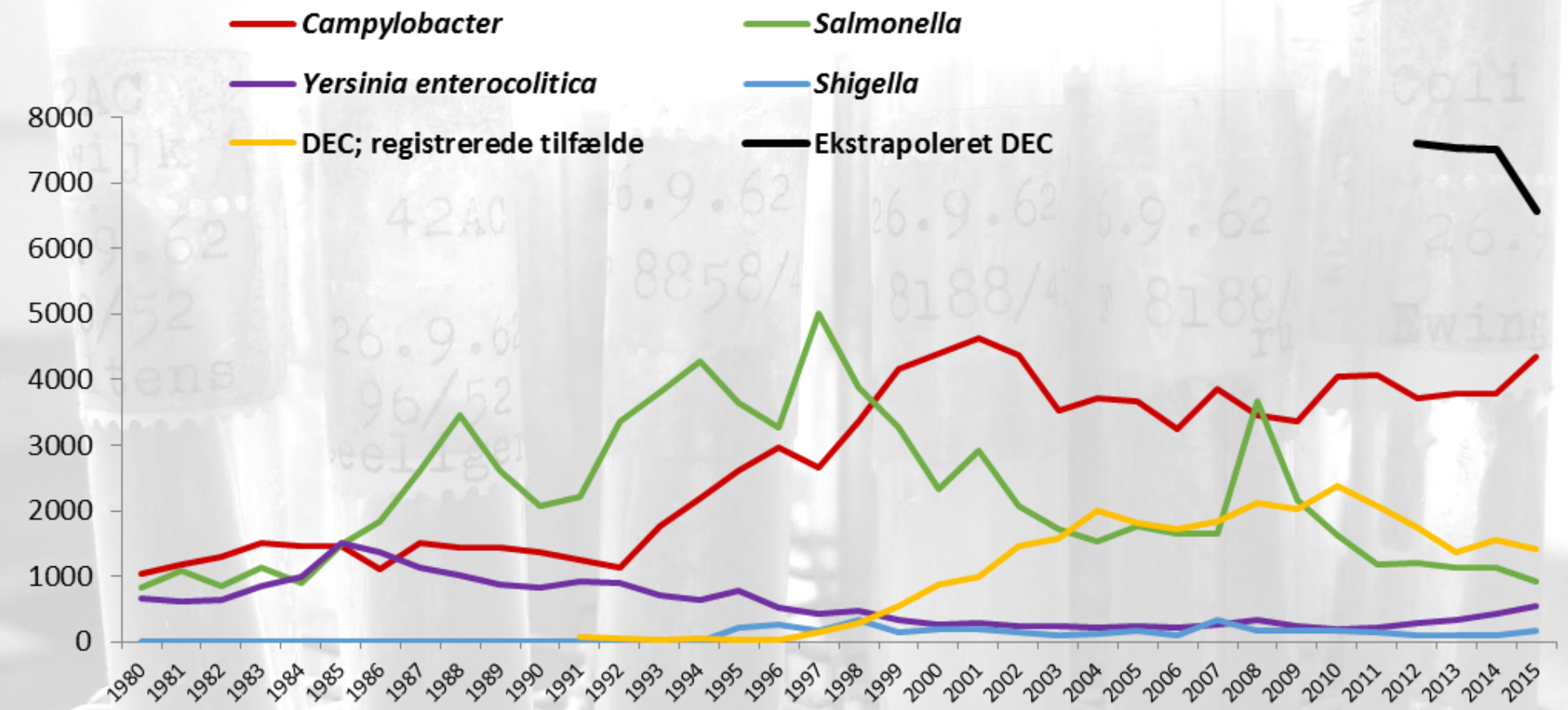


Enteropatogene bakterier: Udviklingen i Danmark 1980- 2015



Enteropatogene bakterier: Udviklingen i Danmark 1980- 2015

DEC = Diarréfremkaldende *E. coli*



Smitsomme sygdomme

- Antibiotikaresistens
- Anmeldelse af smitsomme sygdomme
- Smitsomme sygdomme hos børn
- Coronavirus - MERS-CoV - SARS
- Ebolavirus sygdom
- Flåt og Borrelia
- Fnat
- Hepatitis (leverbetændelse)
- HIV og AIDS
- Influenza
- Klamydia
- Lus
- Lussingesyge - parvovirus
- Meningokoksygdom

VTEC

Opdateret 11. september 2015

VTEC er en særlig coli-bakterie, der kan forårsage diarré (ofte med blod i afføringen). Infektioner med visse VTEC-bakterier kan ledsages af en særlig sygdom, hæmolytisk-uræmisk syndrom (HUS), som er en alvorlig tilstand med bl.a. nyresvigt. HUS forekommer især hos børn efter mavetarm-infektion med visse typer VTEC. VTEC er i reglen en fødevarebåren infektion, men smitte kan også ses ved direkte kontakt til dyr, fra forurenet vand eller som person-til-person smitte.

Tilfælde af VTEC er forbundet med særlige skærpede forholdsregler, og VTEC, herunder HUS, er anmeldelsespligtig. Læs mere:

Sundhedsstyrelsens retningslinjer for håndtering af HUS og VTEC

Vejledning om smitsomme sygdomme hos børn og unge, 2011

Symptomer på VTEC-infektion dækker et spektrum fra kortvarig, ublodig diarré, over svær blodig diarré med voldsomme mavesmerter til alvorlige

RELATERET INDHOLD

- Sygdomstilfælde med sandsynlig smitte fra kebab-restauranter
  21. januar 2015

10. september 2015

STATENS SERUM INSTITUT

Sundhedsstyrelsen

Danish Health and Medicines Authority

Retningslinjer for håndtering af hæmolytisk uræmisk syndrom (HUS) og verocytotoxinproducerende E. Coli (VTEC)

VTEC kan opdeles i henhold til O gruppe/serotype, toksintype samt andre virulensfaktorer. De hyppigst forekommende O grupper i Danmark er O26, O103, O117, O145, O146 og O157 – tilsammen udgør de 64 % af de verocytotoksinproducerende *E. coli*. Sandsynligheden for, at en infektion kompliceres med HUS er primært bestemt af virulensprofilen, og Statens Serum Institut udfører løbende risikovurdering af specifikke VTEC stammers association til HUS. En opdateret oversigt over HUS-associerede VTEC findes på Statens Serum Instituts hjemmeside:
http://www.ssi.dk/Service/Sygdomsleksikon/H/Haemolytisk%20uraemisk%20syndrom.aspx

Hæmolytisk uræmisk syndrom

Hæmolytisk uræmisk syndrom (HUS) er en alvorlig sygdomstilstand, der er karakteriseret ved akut nedsat nyrefunktion, fragmentering af de røde blodlegemer og fald i antallet af blodplader.

I de fleste tilfælde opstår HUS som en komplikation i forbindelse med en bakteriel mave-tarminfektion – ofte med blodig diarré. HUS ses især hos børn med visse typer af verocytotoksin/Shiga toksin producerende *Escherichia coli* (VTEC/STEC) infektion. Andre faktorer kan også medvirke til at udløse HUS, herunder infektion med visse virustyper og brug af visse typer af medicin.

Antallet af tilfælde af HUS ligger typisk mellem 2-7 om året, men har været oppe på 12-16 tilfælde i forbindelse med fødevarebårne udbrud med VTEC; se EPI-nyt 27-33, 2011; og EPI-nyt 45, 2012.

Se overvågningsdata for VTEC - HUS i Danmark.

Søg i Sygdomsleksikon

Søg

Søg

Relateret indhold

E. coli-infektion

Aktuelle EPI-nyt

Nye retningslinjer fra

Efter vtx subtypning er følgende virulensprofiler associeret til HUS (HUSEC):

1. *vtx2a* uanset andre virulensgener
2. *vtx2d* uanset andre virulensgener

Der skelnes i mellem det virulensprofil, som umiddelbart udføres på de fleste klinisk mikrobiologiske afdelinger (KMA'er) og den efterfølgende *vtx* subtypning, der foretages på nogle KMA'er og på SSI. Ved primær påvisning af *vtx2*, uanset andre gener, er der en MULIG klinisk relevant association til HUS (HUSEC) og svar gives som sådan, indtil der er foretaget *vtx* subtypning.

Efter vtx subtypning er følgende virulensprofiler associeret til HUS (HUSEC):

1. *vtx2a* uanset andre virulensgener
2. *vtx2d* uanset andre virulensgener

Meldepligt

Real-Time overvågning af VTEC vhs WGS

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Real-Time Whole-Genome Sequencing for Routine Typing, Surveillance, and Outbreak Detection of Verotoxigenic *Escherichia coli*

Katrine Grimstrup Joensen,[a,b] Flemming Scheutz,[b] Ole Lund,[c] Henrik Hasman,[a] Rolf S. Kaas,[a,c] Eva M. Nielsen,[b] Frank M. Aarestrup[a]

National Food Institute, Division for Epidemiology and Microbial Genomics, Technical University of Denmark, Kongens Lyngby, Denmark[a]; Department of Microbiology and Infection Control, Statens Serum Institut, Copenhagen, Denmark[b]; Center for Biological Sequence Analysis, Department of System Biology, Technical University of Denmark, Kongens Lyngby, Denmark[c]

Fast and accurate identification and typing of pathogens are essential for effective surveillance and outbreak detection. The current routine procedure is based on a variety of techniques, making the procedure laborious, time-consuming, and expensive. With whole-genome sequencing (WGS) becoming cheaper, it has huge potential in both diagnostics and routine surveillance. The aim of this study was to perform a real-time evaluation of WGS for routine typing and surveillance of verocytotoxin-producing *Escherichia coli* (VTEC). In Denmark, the Statens Serum Institut (SSI) routinely receives all suspected VTEC isolates. During a 7-week period in the fall of 2012, all incoming isolates were concurrently subjected to WGS using IonTorrent PGM. Real-time bioinformatics analysis was performed using web-tools (www.genomicepidemiology.org) for species determination, multilocus sequence type (MLST) typing, and determination of phylogenetic relationship, and a specific VirulenceFinder for detection of *E. coli* virulence genes was developed as part of this study. In total, 46 suspected VTEC isolates were characterized in parallel during the study. VirulenceFinder proved successful in detecting virulence genes included in routine typing, explicitly verocytotoxin 1 (*vtx1*), verocytotoxin 2 (*vtx2*), and intimin (*eae*), and also detected additional virulence genes. VirulenceFinder is also a robust method for assigning verocytotoxin (*vtx*) subtypes. A real-time clustering of isolates in agreement with the epidemiology was established from WGS, enabling discrimination between sporadic and outbreak isolates. Overall, WGS typing produced results faster and at a lower cost than the current routine. Therefore, WGS typing is a superior alternative to conventional typing strategies. This approach may also be applied to typing and surveillance of other pathogens.

Bacterial pathogens still pose a major threat to public health, and in order to limit their spread and prevent infectious disease outbreaks, accurate and rapid diagnostics and classification of isolates are of great importance. In current routine practice, isolation and identification are mostly performed at clinical microbiological laboratories, and verification and further characterization are performed for a few selected pathogens at national, or regional, reference laboratories, using a variety of species-specific methods. Typing and surveillance of bacterial pathogens rely mainly on well-established, standardized phenotypic and molecular typing methods, such as serotyping and pulsed-field gel electrophoresis (PFGE) (1, 2). However, to obtain sufficient discrimination between isolates, it is typically necessary to combine typing results from several different typing techniques, both phenotypic and genotypic. As a result, it is laborious, time-consuming, and expensive to perform proper typing for surveillance and outbreak detection.

As the cost of whole-genome sequencing (WGS) has decreased and benchtop sequencing machines enable fast turnaround, it has become increasingly attractive for use in routine diagnostics and typing, and the approach has already been found useful in retrospective outbreak investigations (3, 4).

Although WGS provides detailed information that will, in theory, enable diagnostics and typing solely on the basis of the features in the bacterial genome, it is a challenge to define and extract the appropriate information from the large amount of sequence data that is generated. Thus, to facilitate the use of WGS data for routine diagnostics, typing, and surveillance, it is important that the sequence data can be automatically and quickly converted to clinically relevant information that can be easily interpreted by physicians and public health professionals with limited bioinformatics skills. To achieve this, the Center for Genomic Epidemiology (CGE) provides public, user-friendly web-tools for rapid handling of WGS data and extraction of relevant information, useful for diagnostics, surveillance, and outbreak investigations for the global medical society (www.genomicepidemiology.org).

In this study, as a proof of concept, we demonstrate the usefulness of WGS for routine typing, surveillance, and outbreak detection of verocytotoxin-producing *Escherichia coli* (VTEC) infections in Denmark. VTEC, also known as Shiga toxin-producing *E. coli* (STEC), is a gastrointestinal pathogen, which is typically spread by ingestion of contaminated food or water or person-to-person contact. Rapid and reliable diagnostics and detection of outbreak clusters are of utmost importance for control. VTEC infections cause bloody diarrhea and in some cases hemolytic-uremic syndrome (HUS), which is characterized by kidney failure, thrombocytopenia, and microangiopathic hemolytic anemia, and can be fatal to young children. VTEC pathogenicity is facili-

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COMMENTARY

Whole-Genome Sequencing Data for Serotyping *Escherichia coli*—It's Time for a Change!

Claire Jenkins

Gastrointestinal Bacteria Reference Unit, Public Health England, London, United Kingdom

The accessibility of whole-genome sequencing (WGS) presents the opportunity for national reference laboratories to provide a state-of-the-art public health surveillance service. The replacement of traditional serology-based typing of *Escherichia coli* by WGS is supported by user-friendly, freely available data analysis Web tools. An article in this issue of the *Journal of Clinical Microbiology* (K. G. Joensen, A. M. M. Tetzschner, A. Iguchi, F. M. Aarestrup, and F. Scheutz, J Clin Microbiol, 53:2410–2426, 2015, http://dx.doi.org/10.1128/JCM.00008-15) describes SerotypeFinder, an essential guide to serotyping *E. coli* in the 21st century.

Forskningsnyt

› 2015
› 2014
› 2013
› 2012
› 2011
› 2010

Ny metode til at typebestemme colibakterier

19. august 2015

Forskere fra SSI har udviklet en ny metode, der kan bruges til at type colibakterier. Metoden er udviklet under hensyn til, at information om typer af colibakterier fortsat kan udveksles internationalt, uanset om man bruger traditionelle typningsmetoder eller den ny metode med helgenomsekvensering.

Den traditionelle metode til at typebestemme colibakterier er både langsommelig og dyr. Eftersom helgenomsekvensering er blevet billigere de seneste år, er det oplagt at bruge metoden til typning af colibakterier. Når man laver helgenomsekvensering, får man i løbet af et par dage en stor mængde information om hele bakteriens genom. Og informationerne kommer i et helt andet sprog, end det man har brugt verden over i de sidste 70 år.

Nu har forskere fra Statens Serum Institut offentliggjort et online redskab til brug ved helgenomsekvensering af colibakterier, som gøres tilgængeligt for alle laboratorier verden over. Redskabet, SerotypeFinder og forskningen bag det er præsenteret i det videnskabelige tidsskrift Journal of Clinical Microbiology (JCM).

Ordbog og vejviser til internationalt samarbejde

Søg i Forskningsnyt:

Søg Søg

Relateret indhold

Nationalt WHO Referencelaboratorium for Escherichia og Klebsiella

Kontakt

Mikrobiologi & Infektionskontrol
WHO Collaborating Centre for Reference and Research on Escherichia and Klebsiella
Flemming Scheutz

Tlf.: 3268 3334
Fax: 3268 3231
fsc@ssi.dk

Sygdomsleksikon

E. coli-infektion

JCM
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Escherichia coli O-Genotyping PCR: a Comprehensive and Practical Platform for Molecular O Serogrouping

Atsushi Iguchi,[a] Sunao Iyoda,[b] Kazuko Seto,[c] Tomoko Morita-Ishihara,[b] Flemming Scheutz,[d,e] Makoto Ohnishi,[b] Pathogenic *E. coli* Working Group in Japan

Department of Animal and Grassland Sciences, Faculty of Agriculture, University of Miyazaki, Miyazaki, Japan[a]; Department of Bacteriology I, National Institute of Infectious Diseases, Tokyo, Japan[b]; Division of Bacteriology, Osaka Prefectural Institute of Public Health, Osaka, Japan[c]; Department of Microbiology Infection Control, Statens Serum Institut, Copenhagen, Denmark[d]; WHO Collaborating Centre for Reference and Research on Escherichia and Klebsiella, Statens Serum Institut, Copenhagen, Denmark[e]

The O serogrouping of pathogenic *Escherichia coli* is a standard method for subtyping strains for epidemiological studies and enhancing phylogenetic studies. In particular, the identification of strains of the same O serogroup is essential in outbreak inves-

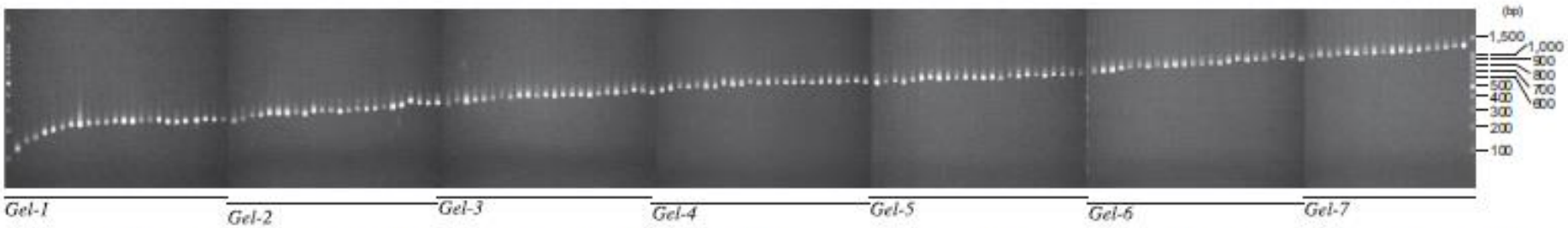
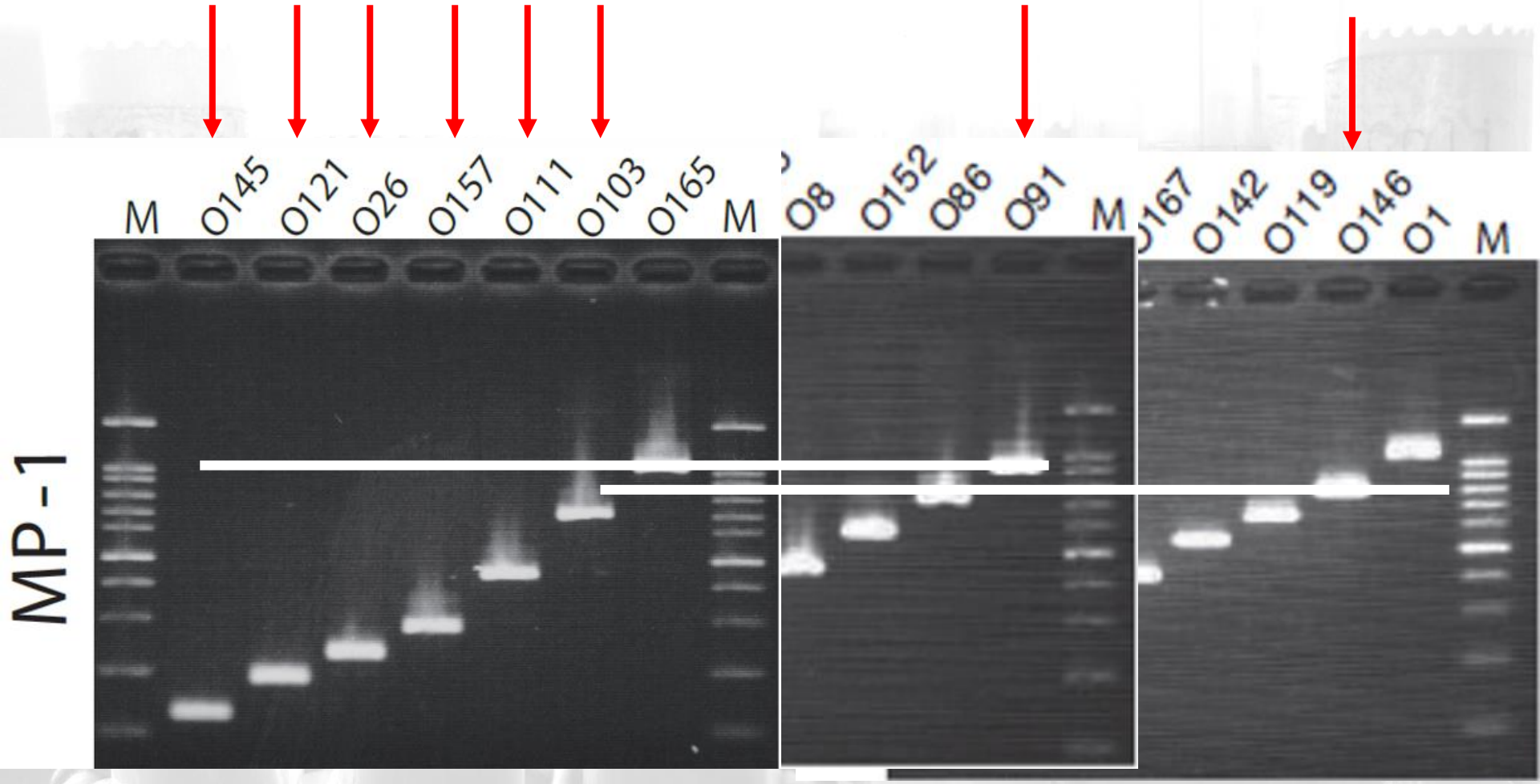
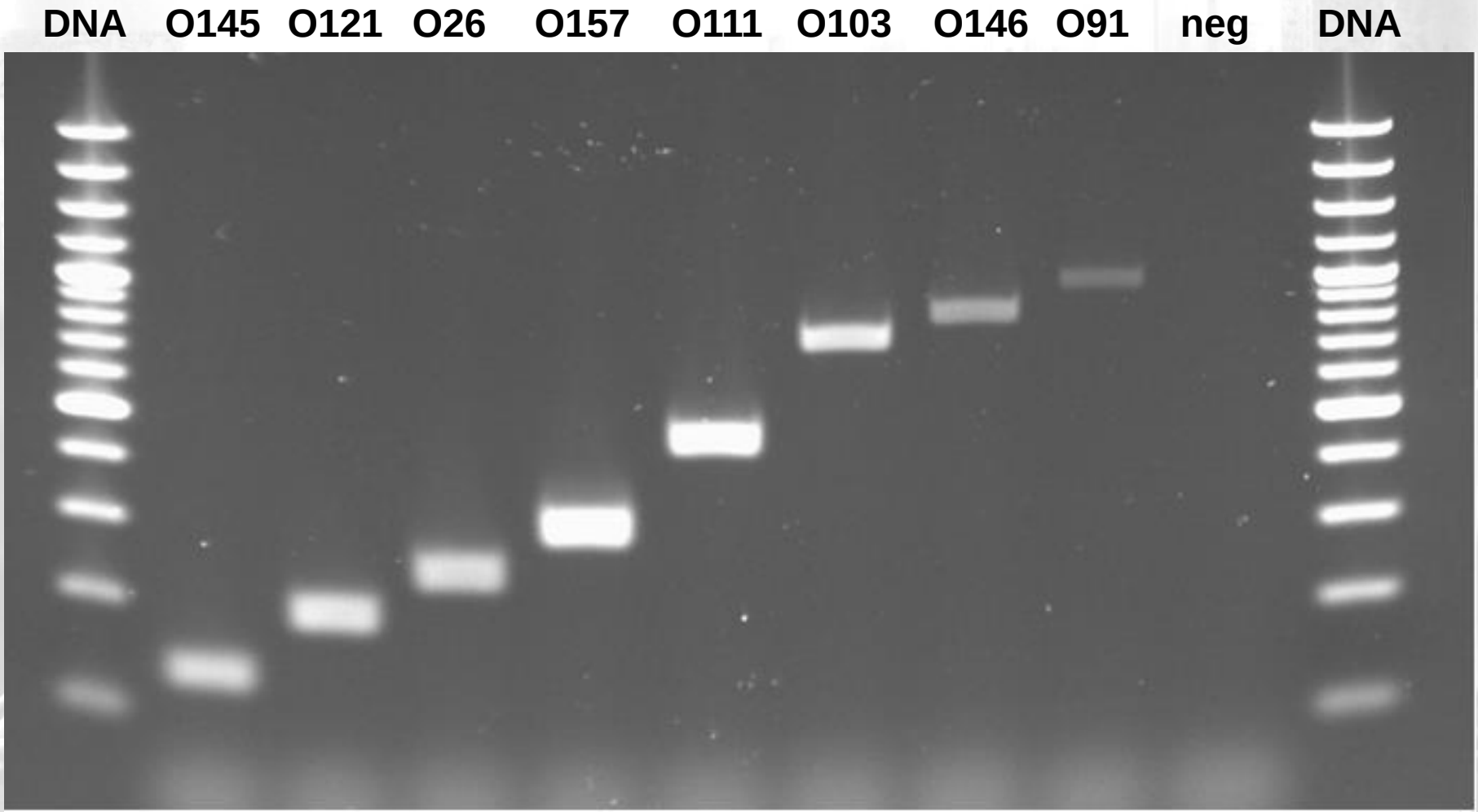


FIG 1 Gel images of 162 PCR products ranging from 132 bp (Og145) to 1,253 bp (Og9). The 162 primer pairs were designed to identify or classify specific *E. coli* O serogroups. Seven gel images were placed in series, and 100-bp DNA ladders were placed at each end.

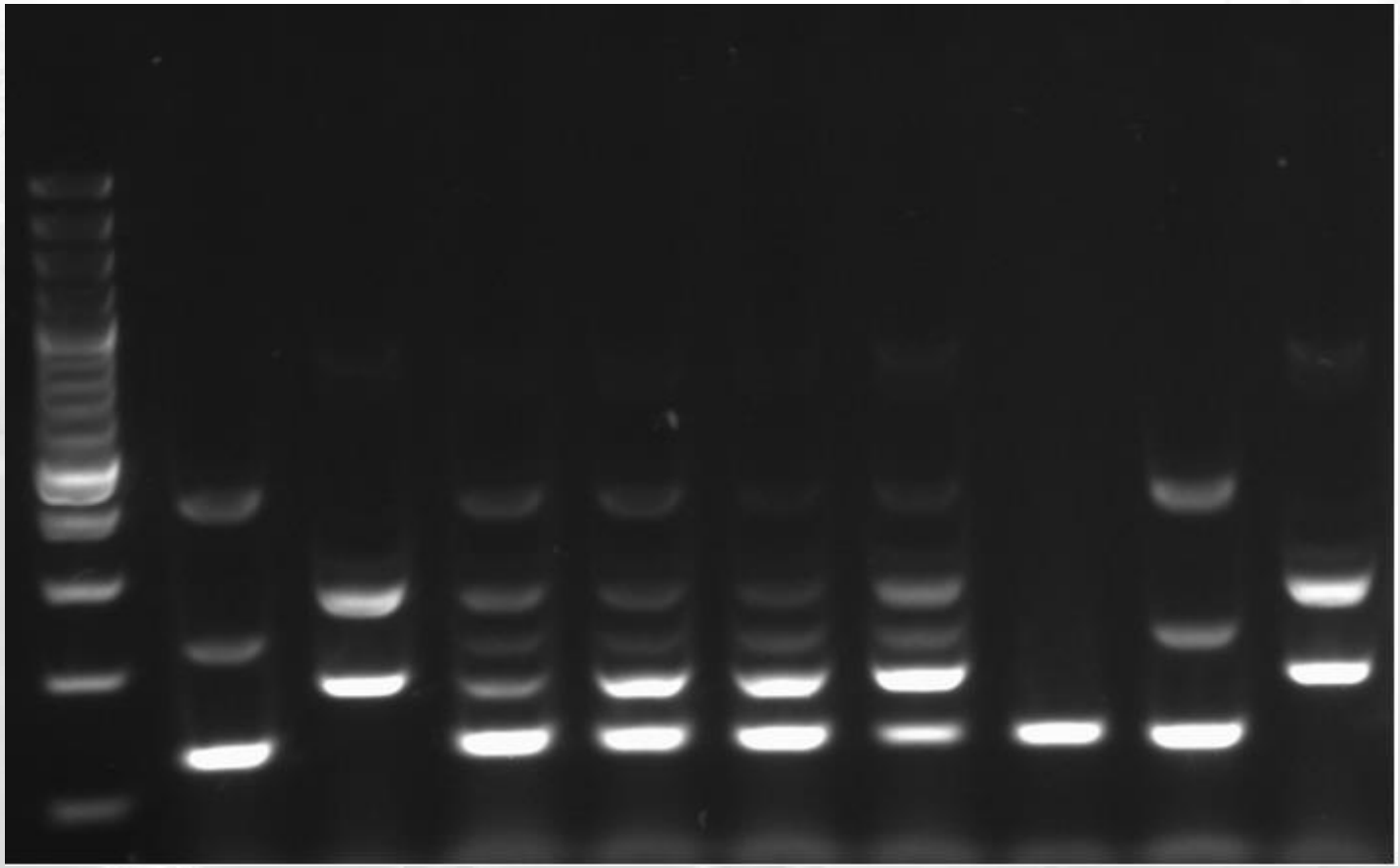
Atsushi's tre relevante mPCR til O gruppering = 53% af VTEC



Første testkørsel med enkeltprimere



Sidste testkørsel med mPCR



O111

O26

O145

Mangler:

O103
O146
og
O91

O157

O121

Nye muligheder for O gruppering

Hvis I kun har DNA

– dvs ingen dyrkning –

kan I indsende DNA

Det kan:

- ***vtx* subtypes**
- **O grupperes for de mest almindelige O grupper**

Workflow – udbrudsdetektion

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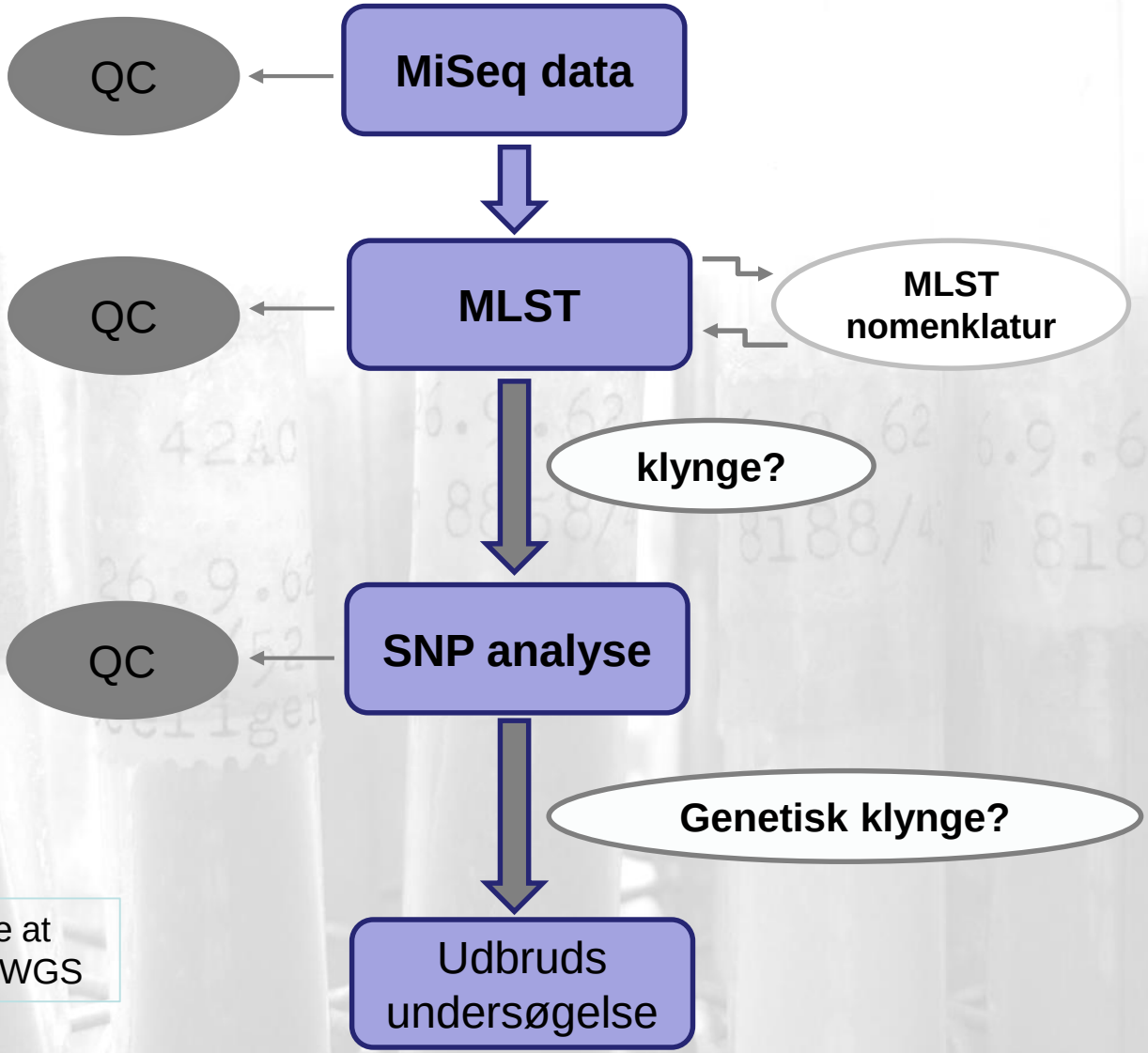
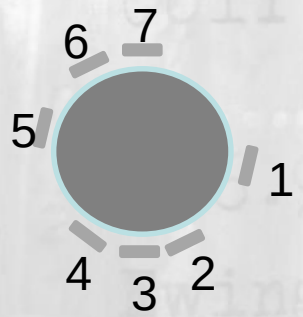
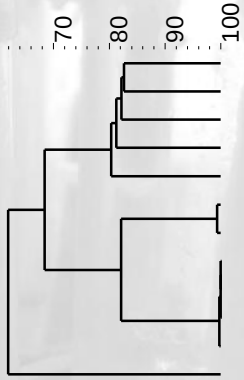
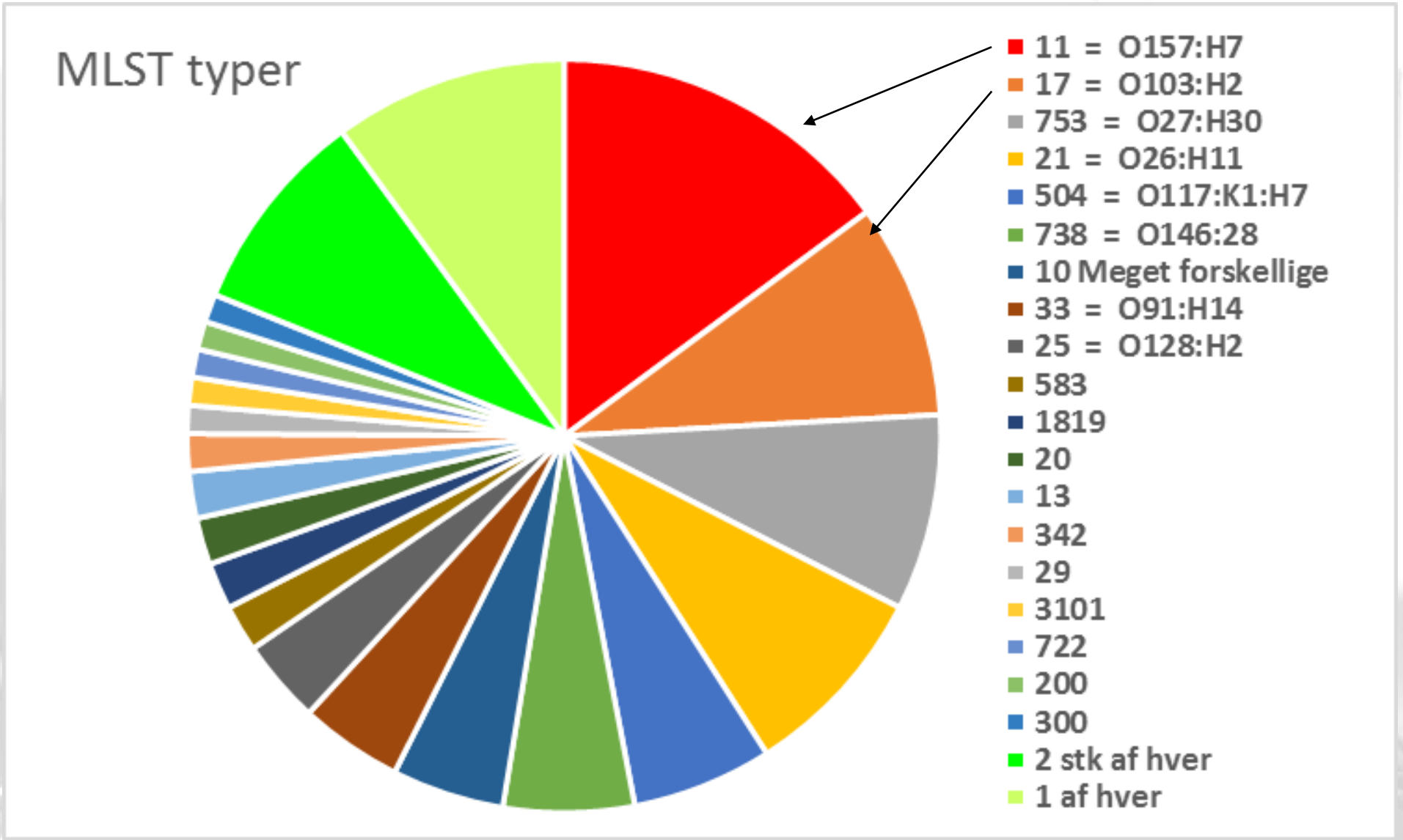






ssi-snp-pipeline at github.com/PHWGS

Multi Locus Sequence Typing = MLST typer (>10 med serotype)



VTEC udbrud med SNP i 2015

10 “genom klynger”!! (Genomic clusters)

- 2-3 personer i hvert udbrud

- **4 institutionsudbrud**
- **2 familieudbrud**
- **4 spredt over tid og sted**

Alle interviewet
Ingen kilder (1 tilfælde dog relateret til kebab udbruddet

Serotyper:

- **O157:H7 (5)**
- **O103:H2 (2)**
- **O26:H11 (2)**
- **O128:H2 (1)**

VTEC er løbende O:H serotypet i hele 2015

116 isolater typet:

- **33 uden fænotype; kun WGS**
- **8 kun fænotype**
- **~85 både fænotype og WGS**

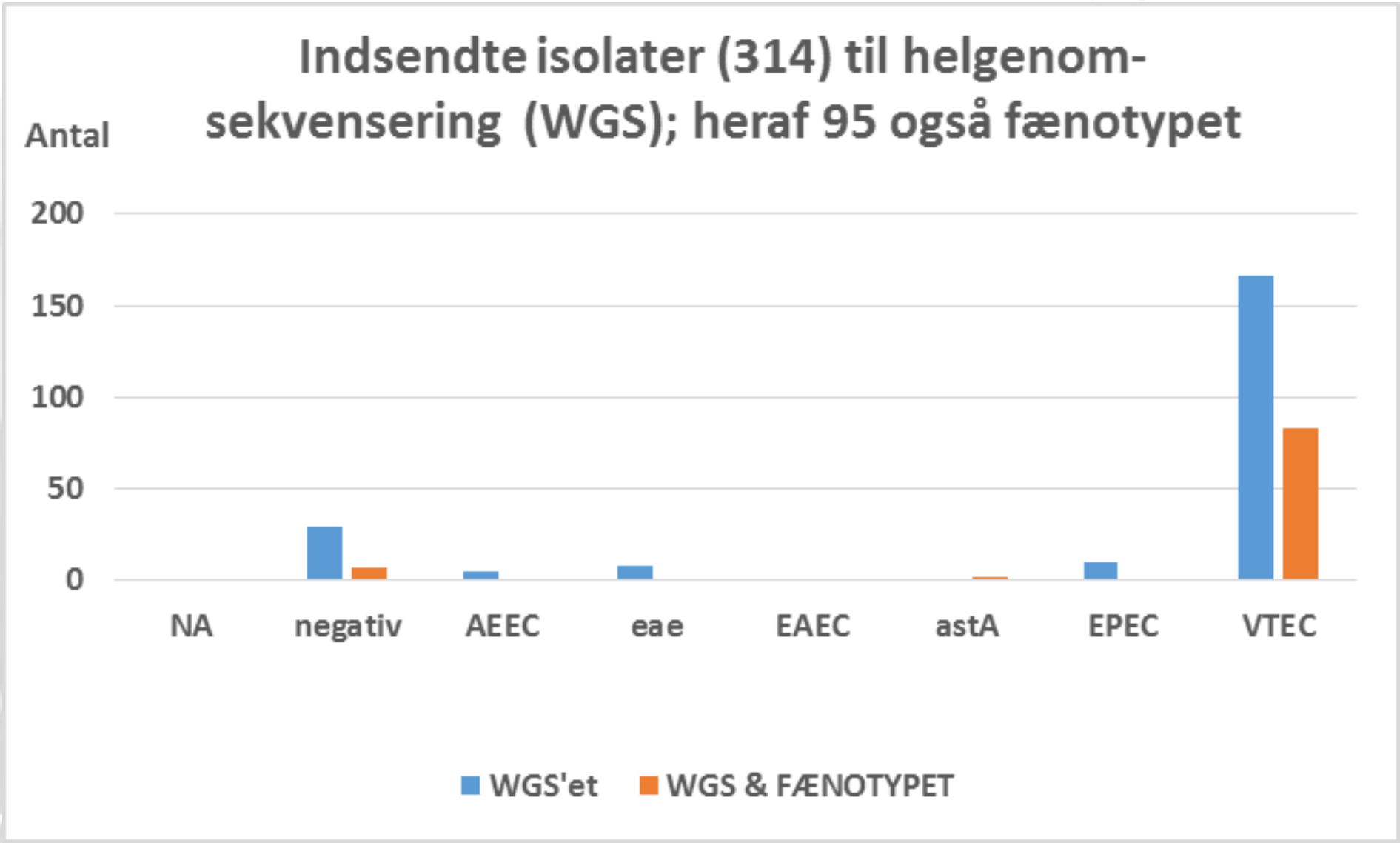
Diskrepanser:

- **1 H**
- **2 O**
- **29 ubevægelige har fået en H type**

Ny viden:

- **O91:H14 (ST 33)**
- **Nye varianter af O40**

STATUS per i går



VTEC serotypning

- **249 isolater WGS'et**
- **Heraf var 31 sekundære isolater**

8 Oru isolater kunne O grupperes:

O 43:H 2	**2**
O 91:H14	**4**
O128:H 2	**1**
O174:H 8	**1**

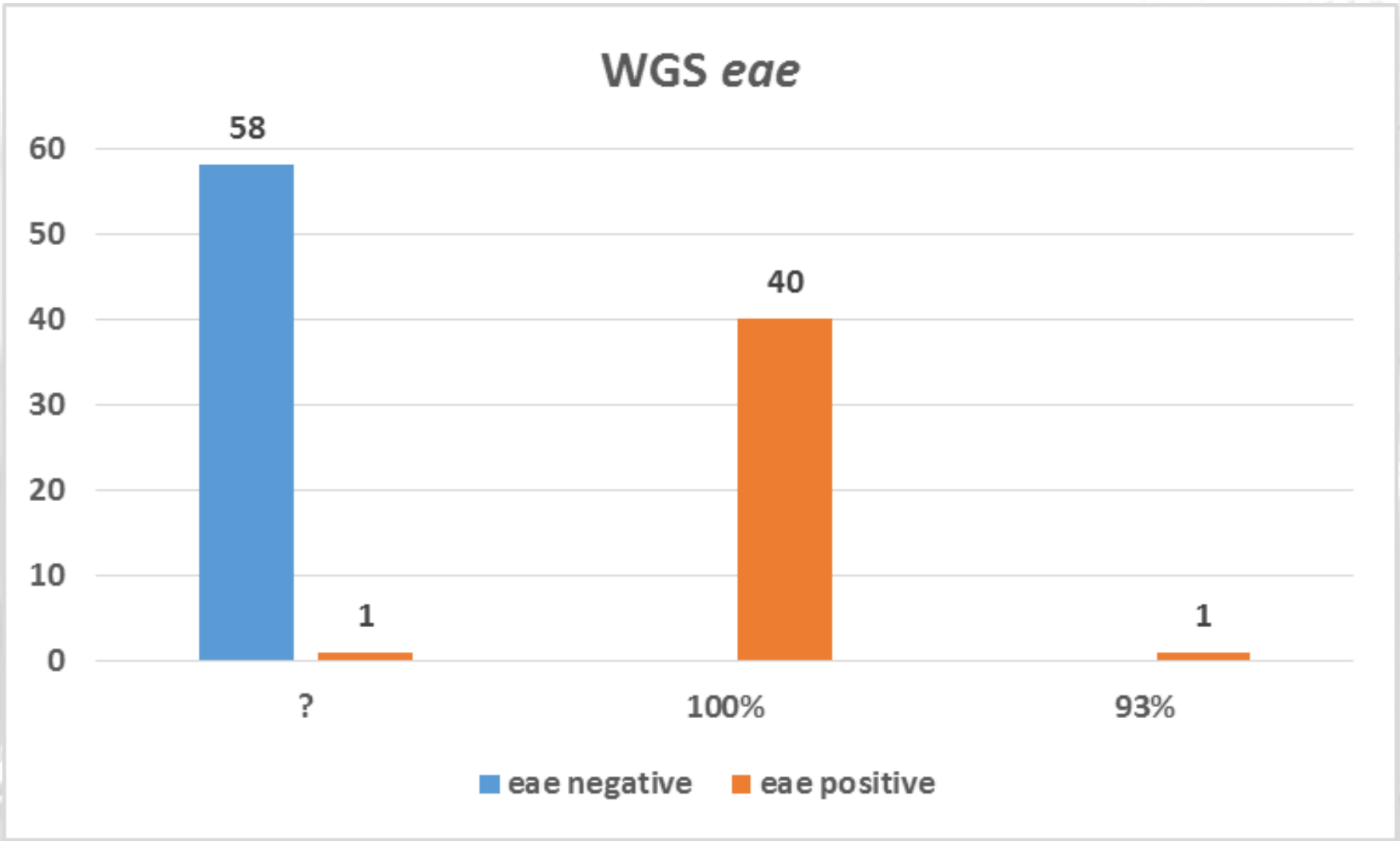
3 Oru isolater kunne ikke O grupperes:

O-:H 4	**1**
O-:H 7	**2**

38 H- (ubevægelige) har fået en H type

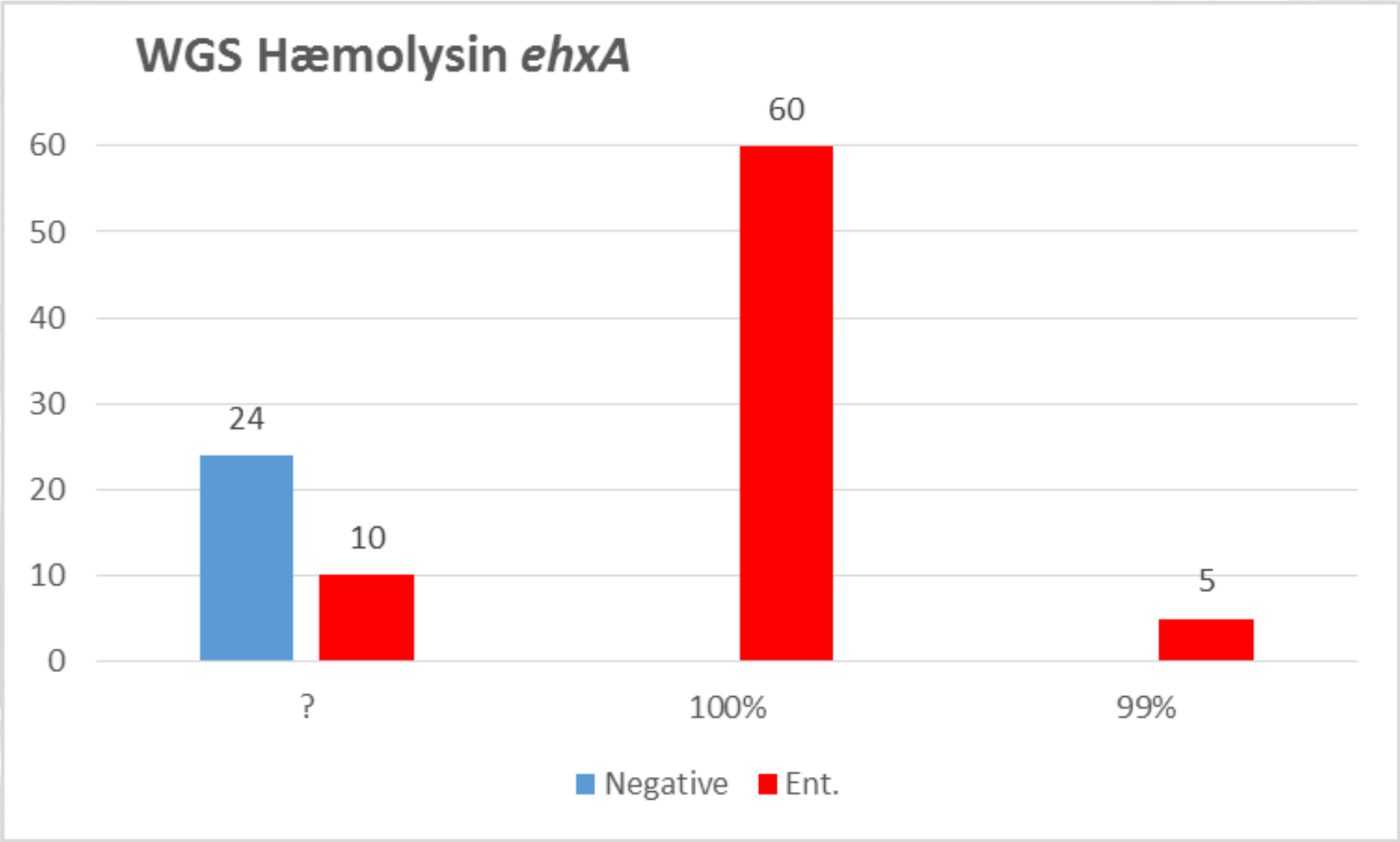
VTEC genotypning

100 isolater WGS'et & dot blottet



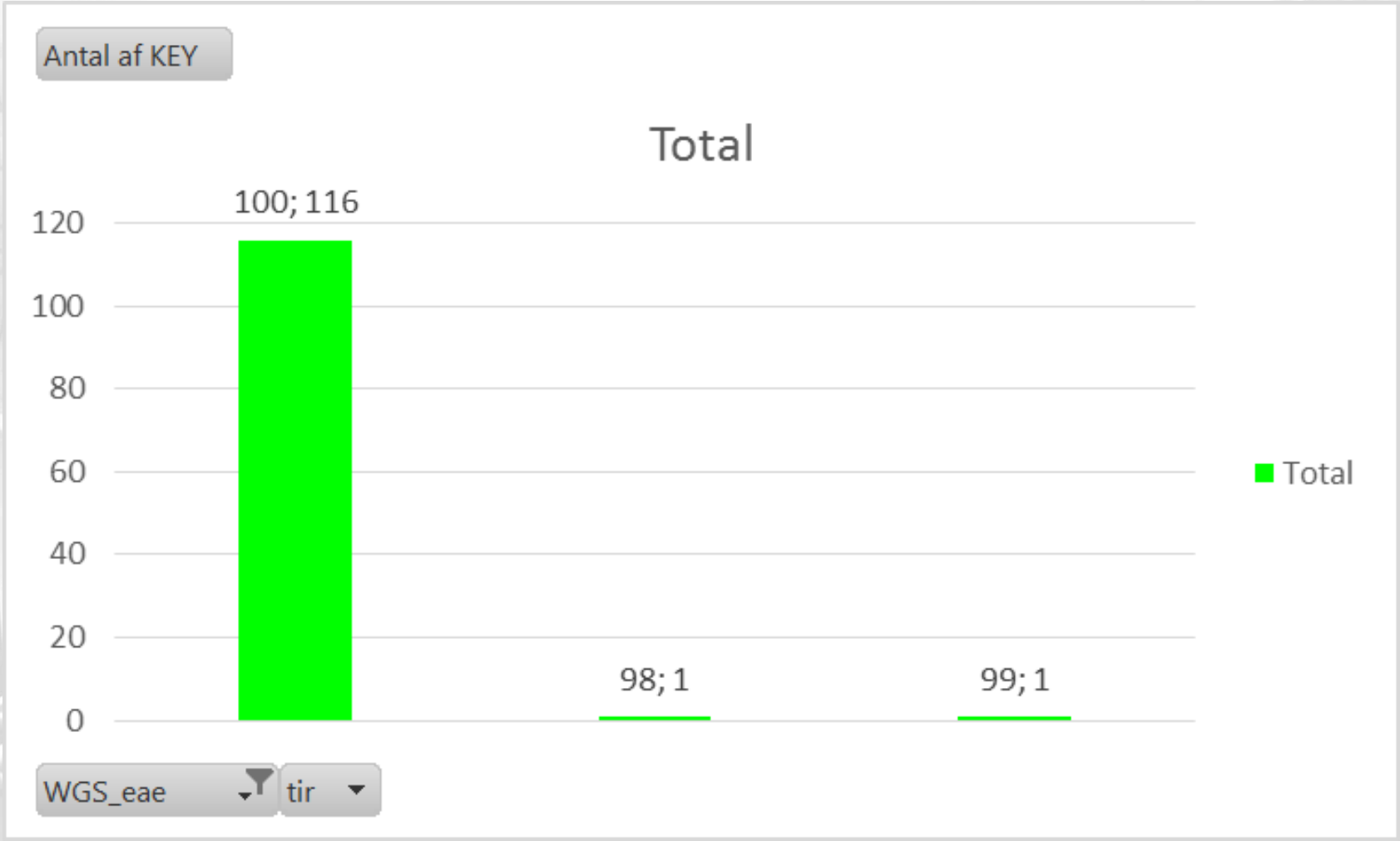
VTEC genotypning

99 isolater WGS'et & fænotypet



VTEC genotypning

118 isolater *eae* (100%) og *tir* = LEE gener



Konklusion

- **SNP analyser af identiske MLST typer finder små (og flere) genetiske klynger, som undersøges ved interview**
- **Disse ser ofte ud til at repræsentere person-til person smitte**
- **I kombination med andre WGS-typnings værktøjer, kan *E. coli* O:H serotypes udelukkende vha WGS-data**
- **WGS er billigere, (hurtigere) og bedre end konventionel typning**

TAK !

”The lab”

Susanne Jespersen

Pia Møller Hansen

Christian Vråby Pedersen

SPECIEL TAK TIL:
CDC, Atlanta, USA

Eva Møller Nielsen
(The boss)

Katrine G. Joensen
(The hard work)

Anna Maria Malberg Tetzschner
(more hard work)

Flemming Scheutz

Atsushi Iguchi
Miyazaki University
Japan

Eva Litrup
(BioNumerics)