Overview of EURL-AMR and NGS activities

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Outline

- EURL-AR presentation and tasks
- EU activities
- Ring trials for E. coli in EURL-AR network
- Overview NGS activities at Research group

The EURL AR is hosted at DTU Food; Research Group of Genomic Epidemiology

- 37 employees in total excluding student helpers
- 4 scientists, 2 lab technicians and 1 IT programmer are directly involved in the EURL AR
- The primary task of the Research Group is to conduct targeted research in order to predict and prevent infectious diseases among people and support the global detection and control with a particular focus on antibiotic resistance and foodborne diseases

The tasks of the EURL-AR

1. Scientific advice and support to the Commission
2. Co-ordination of National Reference Laboratories and provision of technical support
3. Ring trials, comparative testing and quality assurance
4. Confirmatory testing
5. Evaluation and development of analytic methods
6. Missions
7. E-learning

Scientific advice and support to the Commission and others

The Tasks of the EURL-AR

Co-ordination of National Reference Laboratories and provision of technical support

- Creation of the network of NRL's
  - The address list
- Workshops
  - One annual workshop next April 2017
- Training courses aimed at the specific methods to apply in the monitoring (2016 was training on on selective isolation of presumptive ESBL-, AmpC or carbapenemase producing *E. coli* for specific NRL's
- Site visits
- Dissemination of knowledge and information
  - Home page: www.eurl-ar.eu
  - Newsletters
  - E-learning course
- Collection of information on activities at the NRL's
  - Questionnaires

Work of EURL-AR and the monitoring according to EU 652/2013

- Participation in the EFSA Workgroups
- Scientific advice to the EU Commission and participation on the Preparation and discussion of the contents and methods used in the regulation
- Facilitating **training**
- Facilitating **ring trials**
- Development of methods and **confirmatory testing**

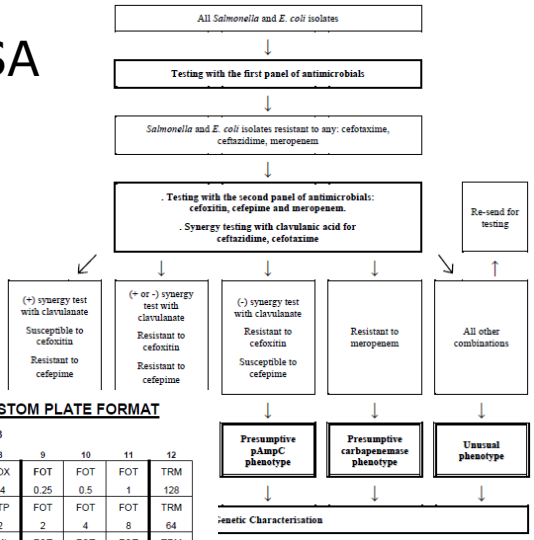
Thus, the activities aim at implementing, from an analytical point of view, the provisions of monitoring of antimicrobial resistance set down the EU regulation 652/2013

Ring trials (EQAS), comparative testing and quality assurance (only EURL, not including WHO and GMI trials)

- Trial 1 (June)
  - *Escherichia coli*
  - Enterococci (incl ID)
  - Staphylococci incl. MRSA
- Trial 2 (October)
  - *Salmonella*
  - *Campylobacter* (incl ID)
  - Genotypic characterization also including detection of ESBL, carbapenem, and AmpC genes
- Trial 3 (October, separately)
  - Matrix EQAS- qualitative detection of ESBL , AmpC or carbapenemase producing *E. coli* from a matrix of caecal and meat samples. The EQAS in 2016 had focus on chicken samples whereas 2017 will be on pig / pork and cattle / beef samples.

Objectives of the EQAS

- To have laboratories evaluate their performance of antimicrobial susceptibility testing (AST)
- To assess the quality of AST in European reference laboratories
- To improve the quality of monitoring data to EFSA
- To identify challenges within the area of AST
- To evaluate the effect of training courses

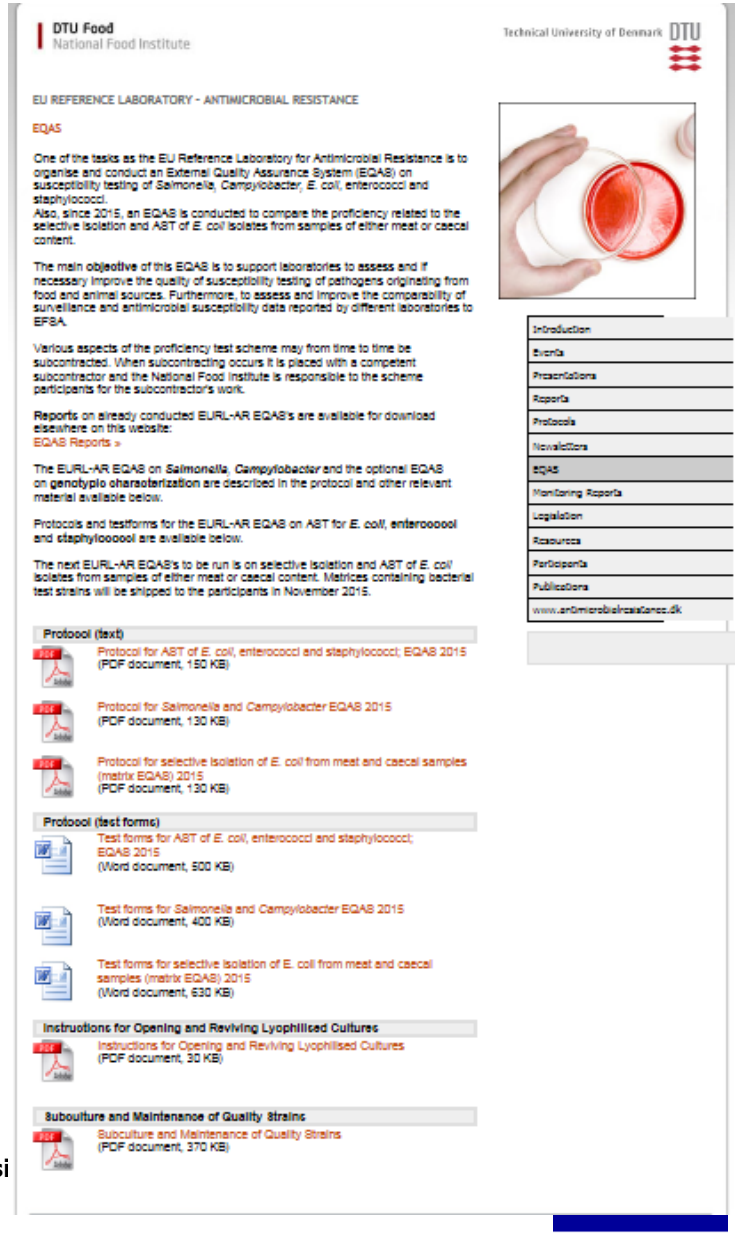


SENSITITRE CUSTOM PLATE FORMAT

Plate Code: EUVSEC2 Date: 30-Oct-13

	1	2	3	4	5	6	7	8	9	10	11	12
A	FOX 0.5	FOX 1	FOX 2	FOX 4	FOX 8	FOX 16	FOX 32	FOX 64	FOT 0.25	FOT 0.5	FOT 1	TRM 128
B	ETP 0.015	ETP 0.03	ETP 0.06	ETP 0.12	ETP 0.25	ETP 0.5	ETP 1	ETP 2	FOT 2	FOT 4	FOT 8	TRM 64
C	IMI 0.12	IMI 0.25	IMI 0.5	IMI 1	IMI 2	IMI 4	IMI 8	IMI 16	FOT 16	FOT 32	FOT 64	TRM 32
D	MERO 0.03	MERO 0.06	MERO 0.12	MERO 0.25	MERO 0.5	MERO 1	MERO 2	MERO 4	MERO 8	MERO 16	TRM 2	TRM 16
E	TAZ 0.25	TAZ 0.5	TAZ 1	TAZ 2	TAZ 4	TAZ 8	TAZ 16	TAZ 32	TAZ 64	TAZ 128	TRM 1	TRM 8
F	FEP 0.06	FEP 0.12	FEP 0.25	FEP 0.5	FEP 1	FEP 2	FEP 4	FEP 8	FEP 16	FEP 32	TRM 0.5	TRM 4
G	F/C 0.06/4	F/C 0.12/4	F/C 0.25/4	F/C 0.5/4	F/C 1/4	F/C 2/4	F/C 4/4	F/C 8/4	F/C 16/4	F/C 32/4	F/C 64/4	POS CON
H	T/C 0.12/4	T/C 0.25/4	T/C 0.5/4	T/C 1/4	T/C 2/4	T/C 4/4	T/C 8/4	T/C 16/4	T/C 32/4	T/C 64/4	T/C 128/4	POS CON

Protocols and relevant documents



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Technical University of Denmark DTU

EU REFERENCE LABORATORY - ANTIMICROBIAL RESISTANCE

EQAS

One of the tasks as the EU Reference Laboratory for Antimicrobial Resistance is to organise and conduct an External Quality Assurance System (EQAS) on susceptibility testing of *Salmonella*, *Campylobacter*, *E. coli*, enterococci and staphylococci.
Also, since 2015, an EQAS is conducted to compare the proficiency related to the selective isolation and AST of *E. coli* isolates from samples of either meat or caecal content.

The main **objective** of this EQAS is to support laboratories to assess and if necessary improve the quality of susceptibility testing of pathogens originating from food and animal sources. Furthermore, to assess and improve the comparability of surveillance and antimicrobial susceptibility data reported by different laboratories to EFSA.

Various aspects of the proficiency test scheme may from time to time be subcontracted. When subcontracting occurs it is placed with a competent subcontractor and the National Food Institute is responsible to the scheme participants for the subcontractor's work.

Reports on already conducted EURL-AR EQAS's are available for download elsewhere on this website:
EQAS Reports »

The EURL-AR EQAS on ***Salmonella***, ***Campylobacter*** and the optional EQAS on **genotypic characterization** are described in the protocol and other relevant material available below.

Protocols and testforms for the EURL-AR EQAS on AST for ***E. coli***, **enterococci** and **staphylococci** are available below.

The next EURL-AR EQAS's to be run is on selective isolation and AST of *E. coli* isolates from samples of either meat or caecal content. Matrices containing bacterial test strains will be shipped to the participants in November 2015.

Protocol (text)

Protocol for AST of *E. coli*, enterococci and staphylococci; EQAS 2015
(PDF document, 150 KB)

Protocol for *Salmonella* and *Campylobacter* EQAS 2015
(PDF document, 130 KB)

Protocol for selective isolation of *E. coli* from meat and caecal samples (matrix EQAS) 2015
(PDF document, 130 KB)

Protocol (test forms)

Test forms for AST of *E. coli*, enterococci and staphylococci; EQAS 2015
(Word document, 500 KB)

Test forms for *Salmonella* and *Campylobacter* EQAS 2015
(Word document, 400 KB)

Test forms for selective isolation of E. coli from meat and caecal samples (matrix EQAS) 2015
(Word document, 630 KB)

Instructions for Opening and Reviving Lyophilised Cultures

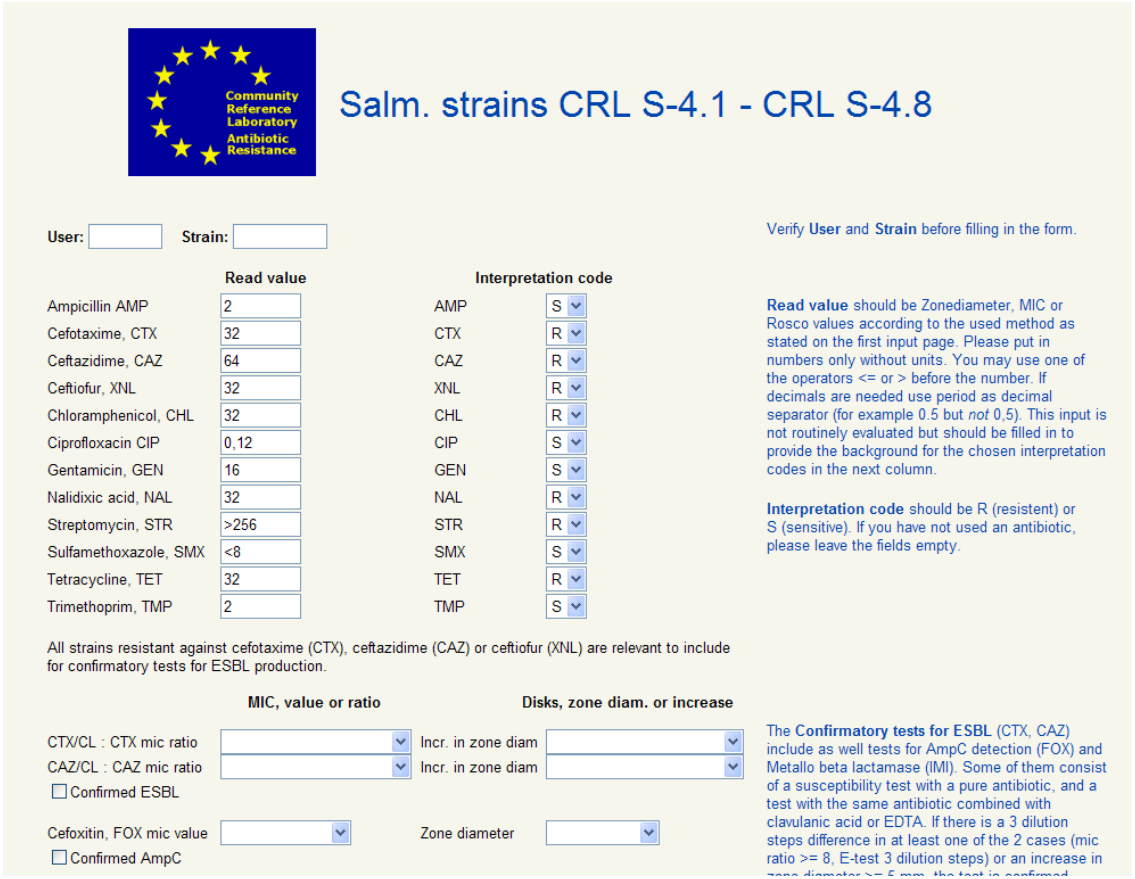
Instructions for Opening and Reviving Lyophilised Cultures
(PDF document, 30 KB)

Subculture and Maintenance of Quality Strains

Subculture and Maintenance of Quality Strains
(PDF document, 370 KB)

The Database

- Results and comments submitted through a web-based data entry program, using individual login
- individual evaluation reports – not instantly due to accreditation
- Summary reports of the performance of the network (for administrators)



Salm. strains CRL S-4.1 - CRL S-4.8

User: Strain:

Verify **User** and **Strain** before filling in the form.

	Read value		Interpretation code
Ampicillin AMP	2	AMP	S
Cefotaxime, CTX	32	CTX	R
Ceftazidime, CAZ	64	CAZ	R
Ceftiofur, XNL	32	XNL	R
Chloramphenicol, CHL	32	CHL	R
Ciprofloxacin CIP	0,12	CIP	S
Gentamicin, GEN	16	GEN	S
Nalidixic acid, NAL	32	NAL	R
Streptomycin, STR	>256	STR	R
Sulfamethoxazole, SMX	<8	SMX	S
Tetracycline, TET	32	TET	R
Trimethoprim, TMP	2	TMP	S

Read value should be Zonediameter, MIC or Rosco values according to the used method as stated on the first input page. Please put in numbers only without units. You may use one of the operators <= or > before the number. If decimals are needed use period as decimal separator (for example 0.5 but *not* 0,5). This input is not routinely evaluated but should be filled in to provide the background for the chosen interpretation codes in the next column.

Interpretation code should be R (resistent) or S (sensitive). If you have not used an antibiotic, please leave the fields empty.

All strains resistant against cefotaxime (CTX), ceftazidime (CAZ) or ceftiofur (XNL) are relevant to include for confirmatory tests for ESBL production.

	MIC, value or ratio		Disks, zone diam. or increase
CTX/CL : CTX mic ratio		Incr. in zone diam	
CAZ/CL : CAZ mic ratio		Incr. in zone diam	
Confirmed ESBL			
Cefoxitin, FOX mic value		Zone diameter	
Confirmed AmpC			

The **Confirmatory tests for ESBL** (CTX, CAZ) include as well tests for AmpC detection (FOX) and Metallo beta lactamase (IMI). Some of them consist of a susceptibility test with a pure antibiotic, and a test with the same antibiotic combined with clavulanic acid or EDTA. If there is a 3 dilution steps difference in at least one of the 2 cases (mic ratio >= 8, E-test 3 dilution steps) or an increase in

The tasks of the EURL-AR

Confirmatory testing and quality assurance

- Confirmatory testing
  - Confirmation of results and characterization of monitoring isolates with WGS

- Evaluation and development of analytic methods
  - Establishing reference strain collections for relevant research and requests
  - Methods for detection of relevant resistances- very focused on the needs in monitoring.

Evaluation and development of analytic methods

- Reference strain collection
  - Available for NRL's on request incl. most relevant ATCC reference strains (original stock)
  - In 2016, to provide a set of new internal reference strains more resistant than the E. coli ATCC 25922 allowing for a better quality assessment of the EUVSEC and EUVSEC2 but also EUVENC and EUCAMP2 MIC plates

- Interpretative criteria
  - Continue the work on the protocols for detection ESBL- or AmpC- or carbapenemase producing E. coli (updates and QC schemes)

- Evaluate procedures and methodologies for quantification ESBL- or AmpC- or carbapenemase producing E. coli

- Collaborate with EUCAST and EFSA to provide missing ECOFF data
- WGS analyses (Campy project, among other)

Workshop's / Training courses

DTU Food
National Food Institute

9th EURL-AR Workshop 2015 Thursday, 23 April – Kgs. Lyngby, Denmark Meeting Room 1, Building 101	
8:45 – 9:00	**Registration, EURL-AR network**
9:00 – 9:10	**Welcome** (Christine Nellemann, Director of DTU Food)
9:10 – 9:20	**Meet and greet and introduction to the day's agenda** (René Hendriksen, EURL-AR)
9:20 – 9:30	**Update from the EURL-AR** (René Hendriksen, EURL-AR)
9:35 – 9:50	**Update from the EU Commission** (Rosa Peran, European Commission)
9:50 – 10:05	**Update from EFSA** (Pierre-Alexandre Beloeil, European Food Safety Authority)
10:05 – 10:25	Coffee break
10:25 – 11:25	**Outcomes of the EURL-AR EQAS 2014 (incl. experiences with the new MIC-panels):** – ***Escherichia coli* and *Salmonella* spp.** (Tomáš Černý, Czech Republic) – ***Enterococcus* spp.** (Gudrun Overesch, Switzerland) – ***Staphylococcus* spp. and methicillin-resistant *Staphylococcus aureus* (MRSA)** (Irena Zdovc, Slovenia) – ***Campylobacter* spp.** (Lurdes Clemente, Portugal) – **Genotypic characterization, ESBL-genes** (Cristina de Frutos Escobar, Spain)
11:25 – 11:35	***Salmonella/Campylobacter* EQAS results – evaluation of MIC-values** (Susanne Karlsmose, EURL-AR)
11:35 – 12:10	**General discussion, EQAS,** incl. Lina will give info about the upcoming 'Matrix EQAS' (qualitative detection of ESBL and AmpC producing *E. coli* from a matrix of caecal and food samples (cattle and swine / beef and pork))
12:10 – 13:10	**Lunch** ('glassalen')
13:10 – 14.00	**Some ECOFFs are missing; how do we define them?** (Gunnar Kahlmeter, EUCAST)
14:00 – 14.20	**Outcome of 2014 survey on NRL's regarding the participation in EURL-AR network activities;** brief presentation by Susanne Karlsmose, EURL-AR
14:20 – 14:30	**Introduction to E-learning on antimicrobial resistance** (Lina Cavaco, EURL-AR)
14:30 – 14.45	**AMR monitoring at slaughter for *Campylobacter* and AmpC-, ESBL- and carbapenemase-producing *Salmonella* and *E. coli* – experiences from an NRL in 2014** (Antonio Battisti, Italy)
14:45 – 15.45	**Specific monitoring of ESBL- or AmpC- or cabapenemase-producing *E. coli*; experience with Decision 2013/652/EU** (discussion groups and coffee break)
15:45 – 16.15	Plenum follow-up

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DTU Food
National Food Institute

9th EURL-AR Workshop 2015 – Joint day with FWD network Friday, 24 April – Kgs. Lyngby, Denmark Meeting Room 1, Building 101	
8:45 – 9:00	**Registration, FWD network**
9:00 – 9:20	**Update from the EU Commission** (Rosa Peran, European Commission) **Update from the ECDC-FWD** (Therese Westrell, ECDC)
9:20 – 9:25	**Introduction to the day's agenda** (Rene Hendriksen, DTU Food, Denmark)
9:25 – 9:55	**AST of *Salmonella* spp. and *Campylobacter* - summary of NRL-AR- EQAS** (Susanne Karlsmose, DTU Food, Denmark) **and ECDC-FWD-EQAS** (Mia Torpdahl, SSI, Denmark)
9:55 – 10:20	**Antimicrobial resistance genes in monitoring programmes, a review** (Frank Aarestrup, EURL-AR, Denmark)
10:20 – 10:45	Group picture; coffee break
10:45 – 11:15	**ISO and CLSI standardization of resistance genes** (Jean Patel, CDC, Atlanta, US)
11:15 – 11:25	**Discussion**
11:25 – 11:55	**Introduction to the WHO Global Action Plan (GAP)** (Hilde Kruse, WHO EURO)
11:55 – 12:15	**FAO-activities in relation to AMR and in relation to the WHO Global Action Plan (GAP)** (Henk Jan Ormel, FAO))
12:15 – 12:25	**Discussion**
12:25 – 13:25	**Lunch** ('glassalen')
13:25 – 14:00	**ESBL-project in Sweden; report published in 2014** (Sara Byfors, Sweden)
14:00 – 14:35	**Outcomes of the work with the JIACRA report** (Pierre-Alexandre Beloeil, European Food Safety Authority; Dominique Monnet, ECDC)
14:35 – 15:15	**National presentation, country #1** (TBD) **National presentation, country #2** (TBD) **Discussion**
15:15 – 15:35	**Plenum discussion and AOB**
15:35 – 15:45	**Future perspective and closing remarks** (René Hendriksen, DTU Food, Denmark; Dominique Monnet, ECDC)

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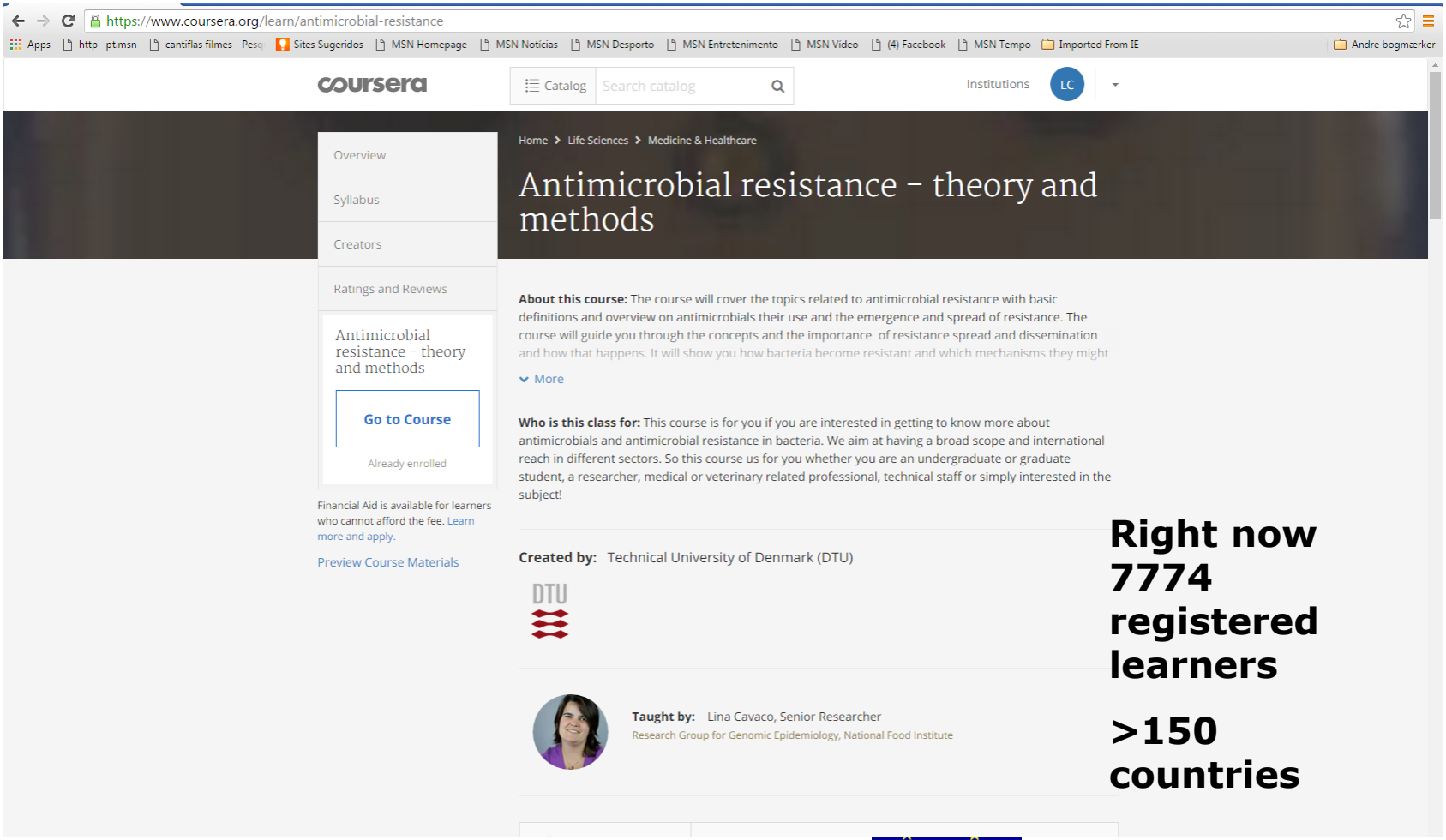
Missions for specific assistance to individual laboratories (site visits)

- Some NRL's and 3rd countries such as candidate countries might have a need for special assistance in the implementation of the EC action plan on AMR
  - 1 - 2 visits per year since 2006
  - 3 days agenda – lab / theoretical lectures
  - Summary report shared with MS and EC
  - Follow up on corrective actions

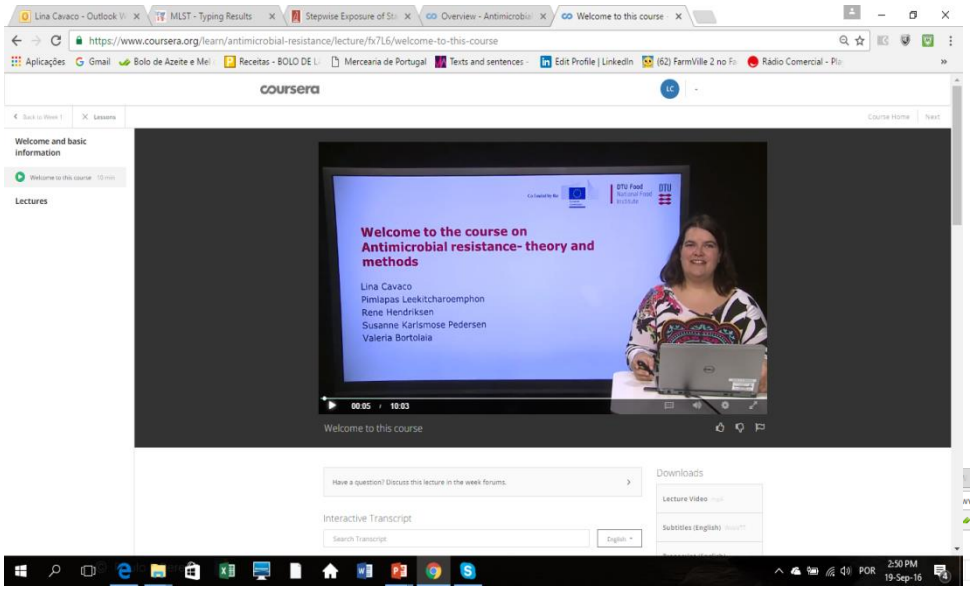
E-learning :
https://www.coursera.org/learn/antimicrobial-resistance

Course page with course contents and information:

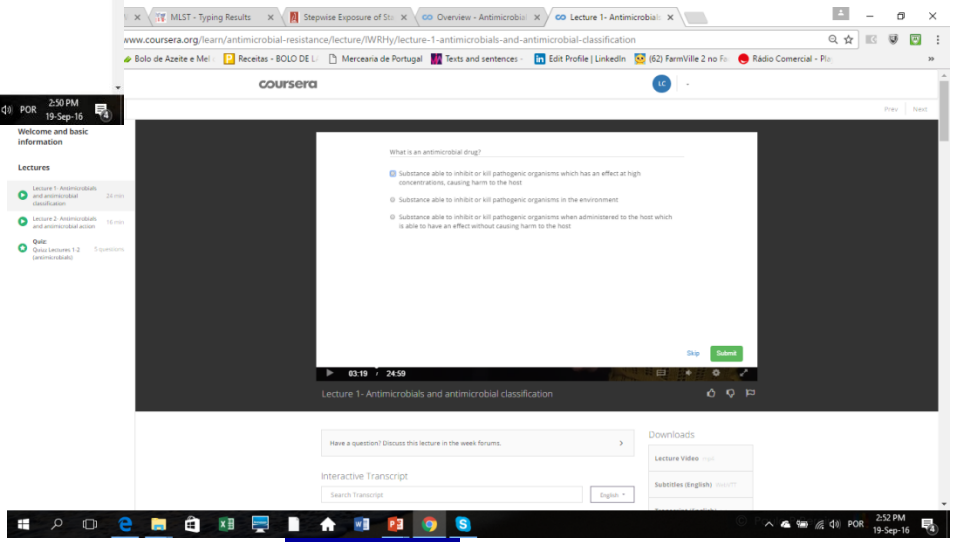


Learning Materials

Video lectures



With quizzes, discussion forums, etc



Quality Assurance of the EQAS

- Accredited methods (relevant for the EQAS):
  - 'MIC testing' according to EN/ISO 17025
  - 'Provider of EQAS (serotyping and AR)' according to ILAC G13 (International Laboratory Accreditation Cooperation)
- International standards/guidelines
  - ISO (International Organization for Standardization)
  - CLSI (Clinical and Laboratory Standards Institute)
  - EUCAST (The European Committee on Antimicrobial Susceptibility Testing – www.eucast.org)

Use of WGS data …

- Data can be used to look look for a number of species through DNA identification, confirm species of isolates, look for new res/ virulence genes and other known factors and several typing methods
- Very quick response (thousands of isolates run in short time)
- Look for genes that relate to previously known genes (%)
- Possibilites of taking findings into functional studies (assays)

Relevant projects/Activities ongoing at our Research group

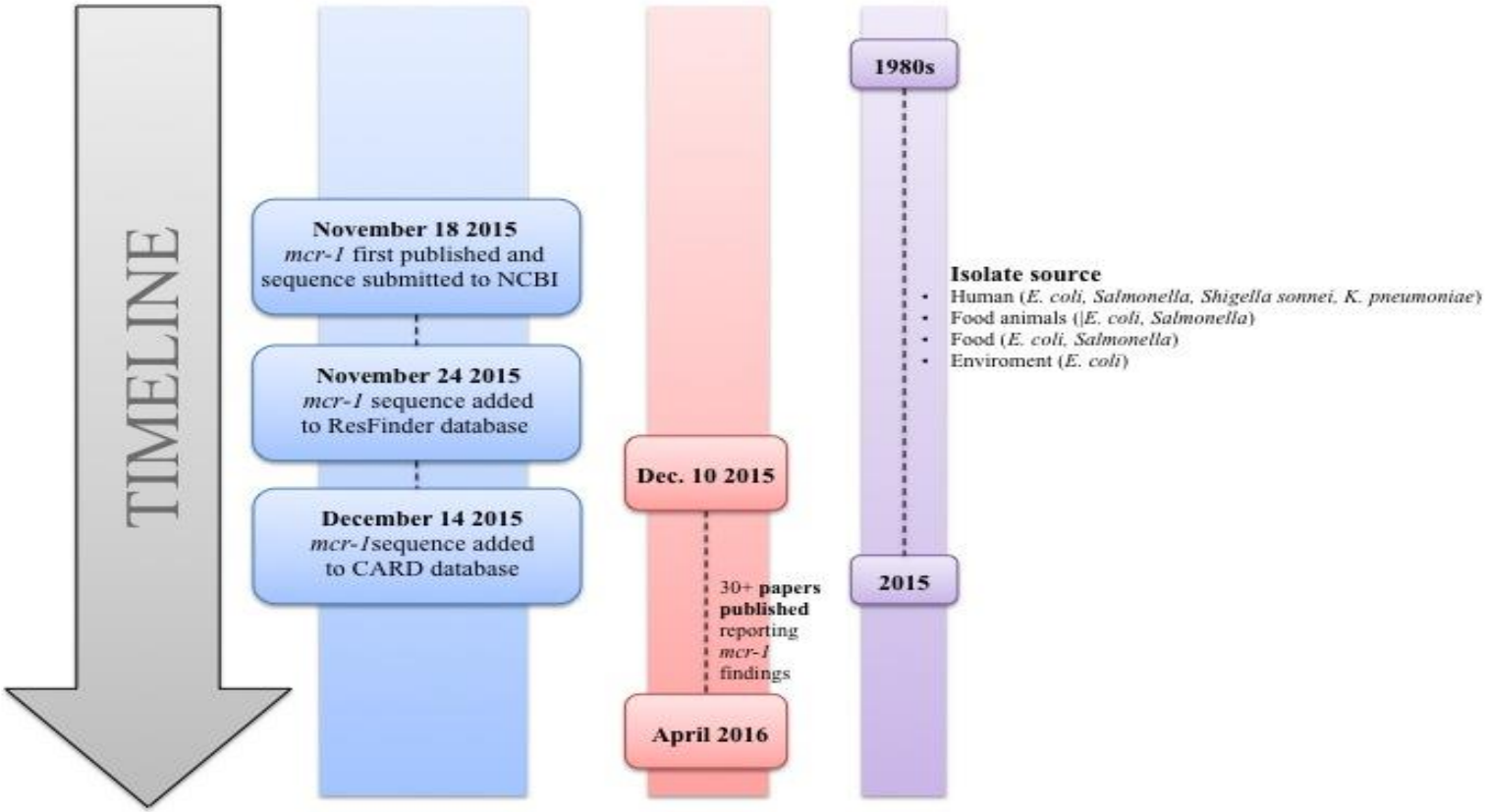
- **WHO AMR** and **WHO CC-** Training and proficency testing including serotyping Salmonella, AST of Salmonella and Campy and as part of GMI and WHO CC proficency testing of WGS
  - Dry lab – data analysis
  - DNA samples- sequencing
  - Isolates

- **COMPARE- EUROPE** – Horizon 2020 project aiming at data sharing and quick detection of outbreaks. aim to share knowledge across time and worldwide.

- **ENGAGE-** project aimed at training and enahnce capacities of WGS in EU- EFSA funded- include training as twinning and courses and proficiency testing

- **EFFORT-** Project lead by Utrecht university and focused on AMR in animals and food chain farm to fork approach- using metagenomics

WGS data and data share makes things fast and global!!!



June/ July- guidance at EU / WHO level

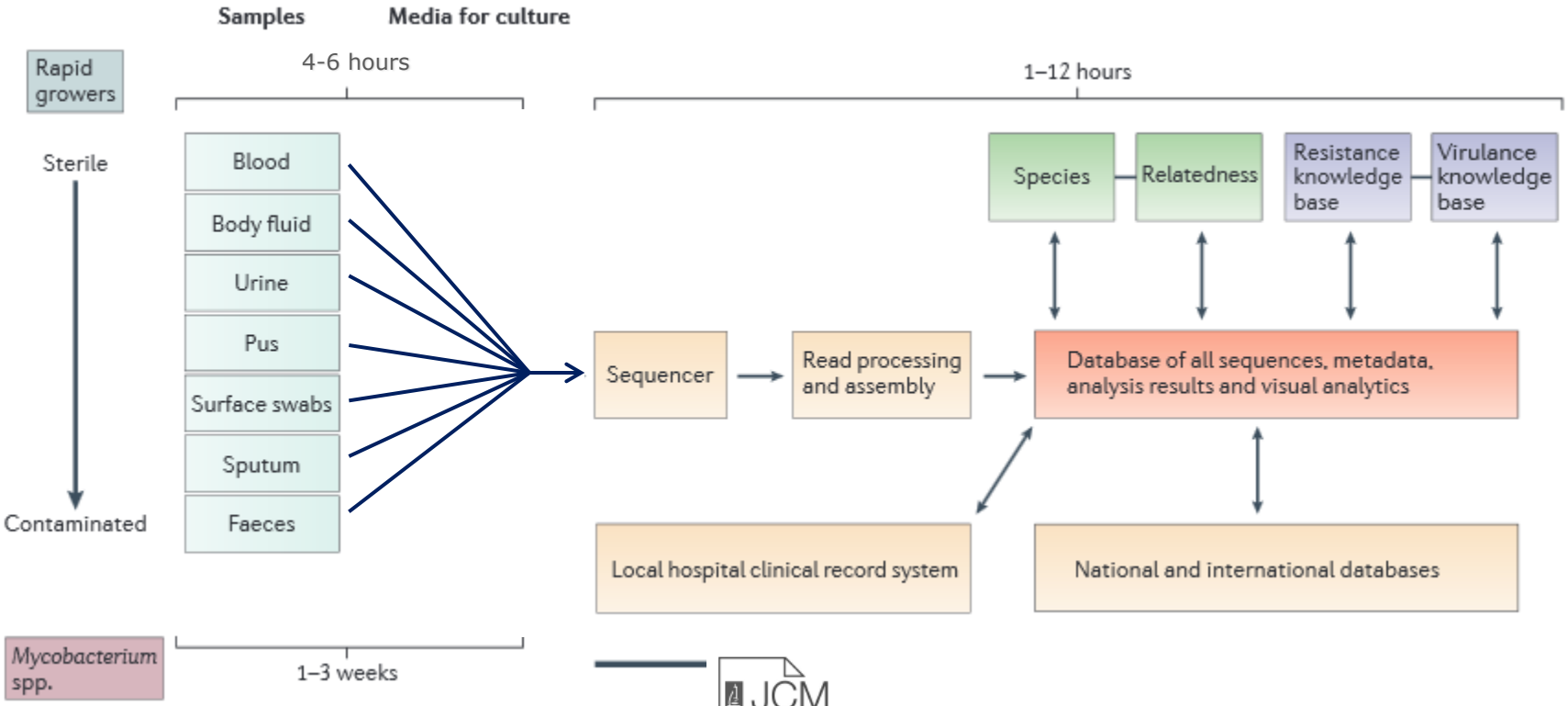
CGE tools

- Species Identification (16S and Kmer finder)
- Virulence finder
- Serotype finder
- Plasmid finder
- Typing and phylogeny (in silico serotyping, MLST, pMLST, plasmid replicon typing, SNP analysis tools CSI phylogeny…
- Single service and batch upload tools to analyse own data (upload metadata together with DNA sequences)
- My Database finder- to find other sequences not included in the remaining tools
- Metagenomic tools- MG mapper…

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

Future School

No isolation required!



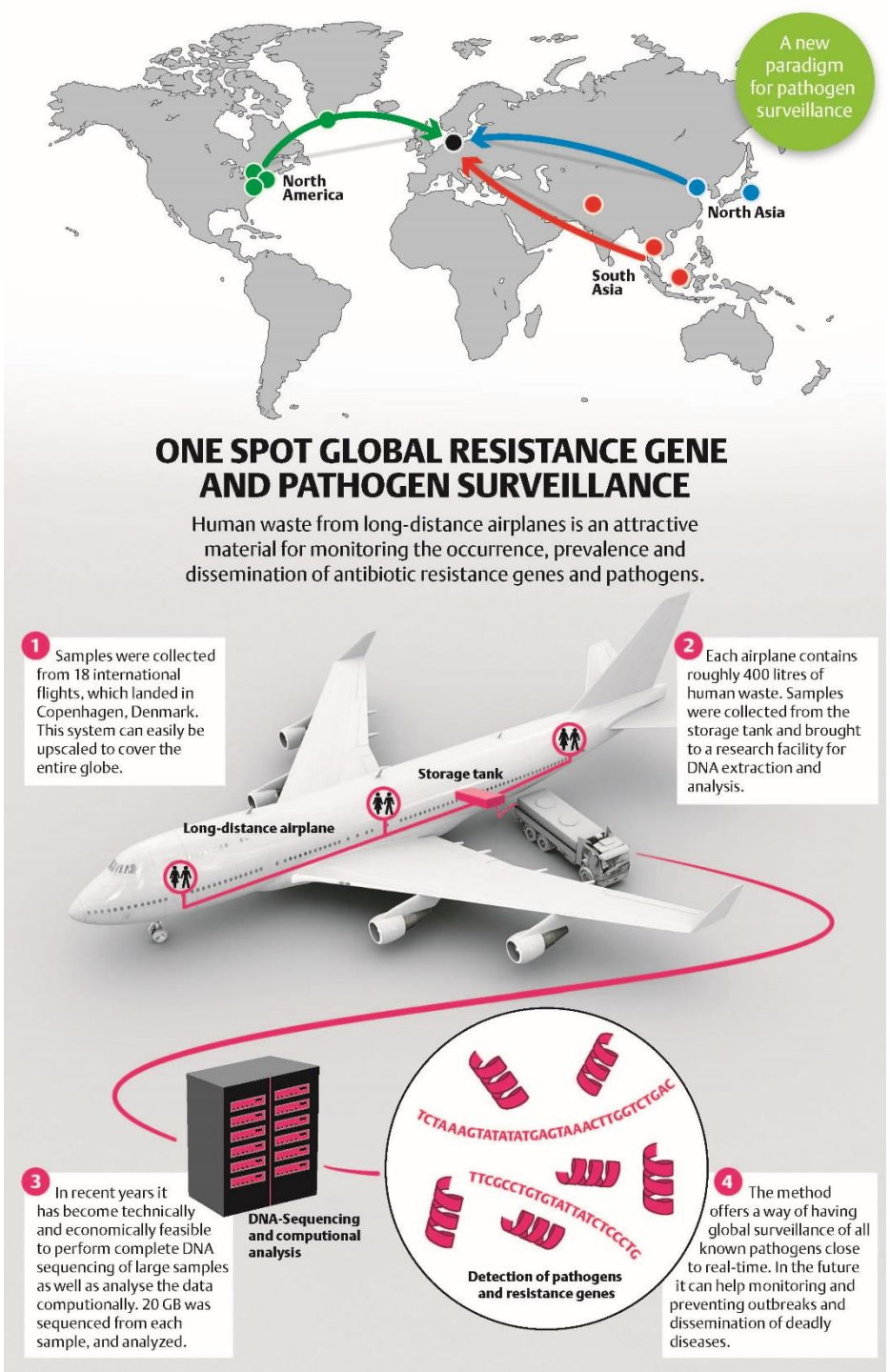
Modified from Didelot *et al.*, 2012.

JCM
Journals.ASM.org

Rapid Whole-Genome Sequencing for Detection and Characterization of Microorganisms Directly from Clinical Samples

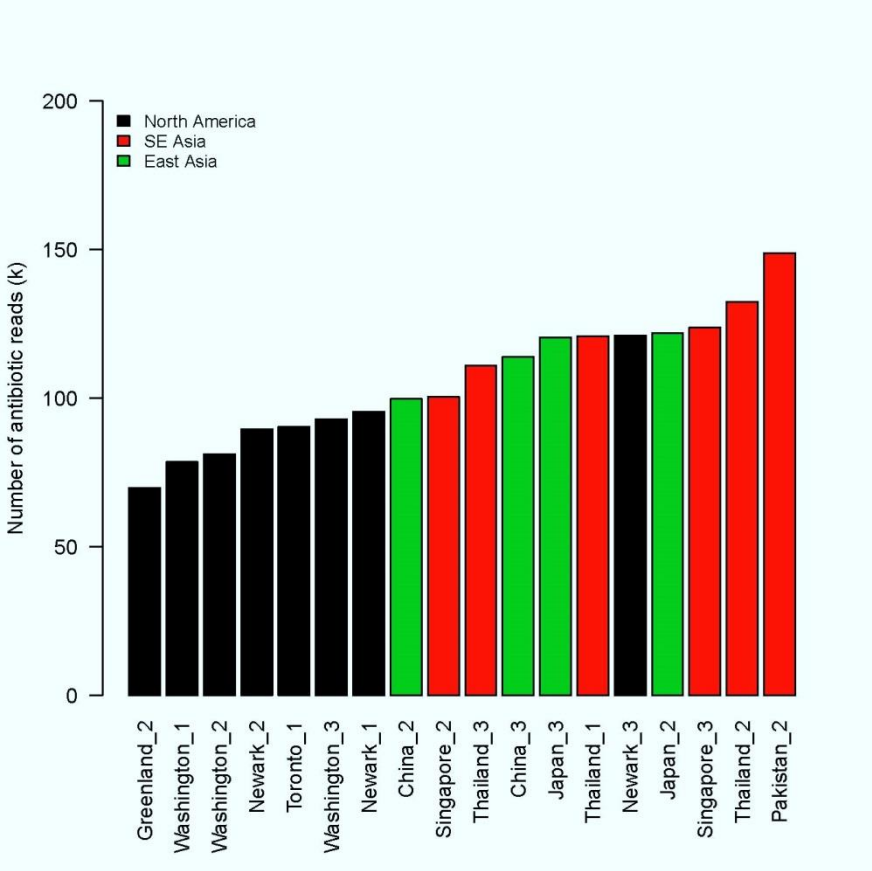
Henrik Hasman,[a] Dhany Saputra,[b] Thomas Sicheritz-Ponten,[b] Ole Lund,[b] Christina Aaby Svendsen,[a] Niels Frimodt-Møller,[c] Frank M. Aarestrup[a]

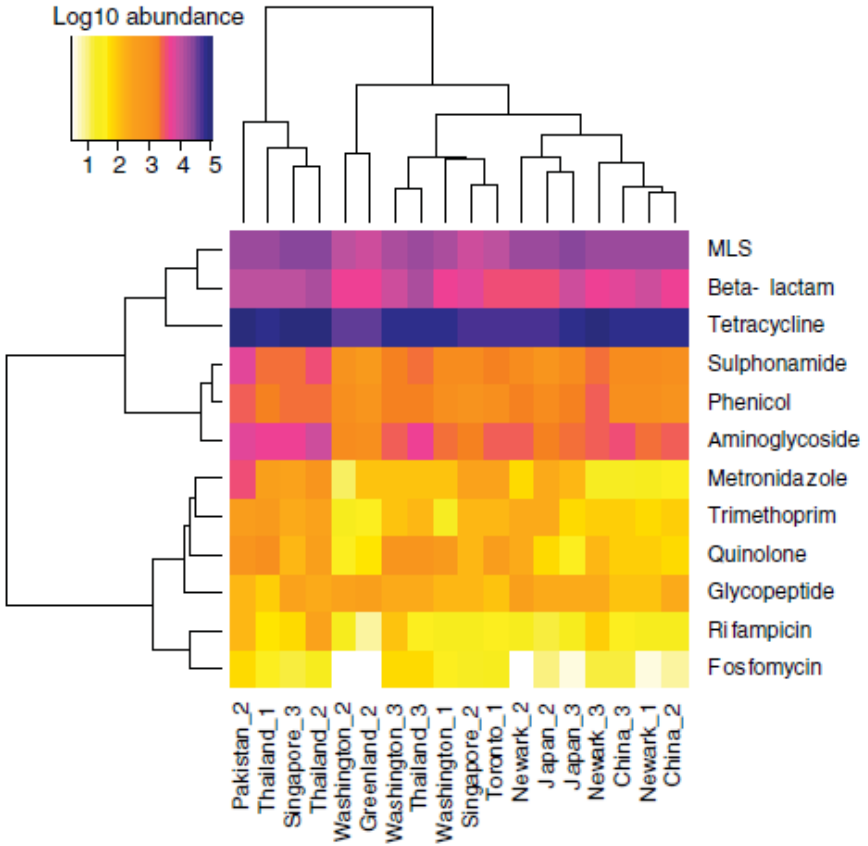
National Food Institute, Technical University of Denmark, Lyngby, Denmark[a]; Systems Biology, Technical University of Denmark, Lyngby, Denmark[b]; Hvidovre Hospital, Hvidovre, Denmark[c]



Petersen, TN, Rasmussen, S, Hasman, H, Carøe, C, Bælum, J, Schultz, AC, Bergmark, L, Svendsen, CA, Lund, O, Sicheritz-Pontén, T & Aarestrup, FM 2015, 'Meta-genomic analysis of toilet waste from long distance flights; a step towards global surveillance of infectious diseases and antimicrobial resistance' *Scientific Reports*, vol 5, 11444., 10.1038/srep11444

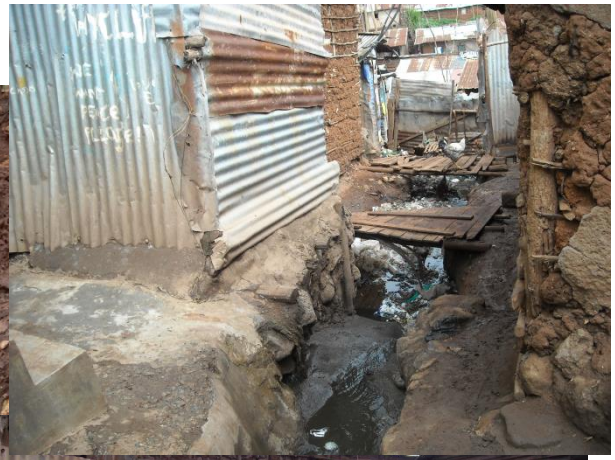
Metagenomics analysis –
Quantification of all bacterial and virus including AMR genes for surveillance

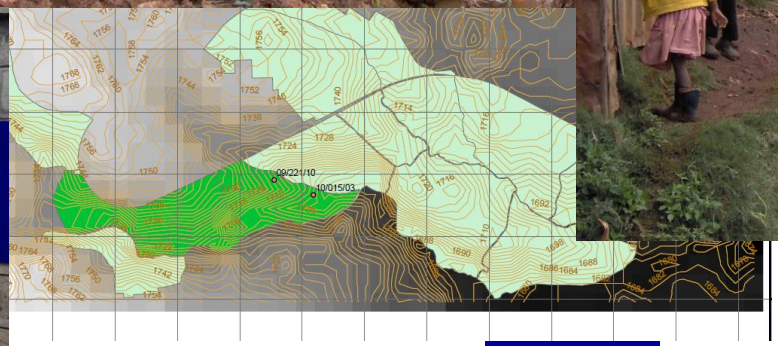




Norda L, Petersen T et al. 2015. Sci Rep.

Disease hotspot surveillance - Slumcity of Kibera in Nairobi, Kenya

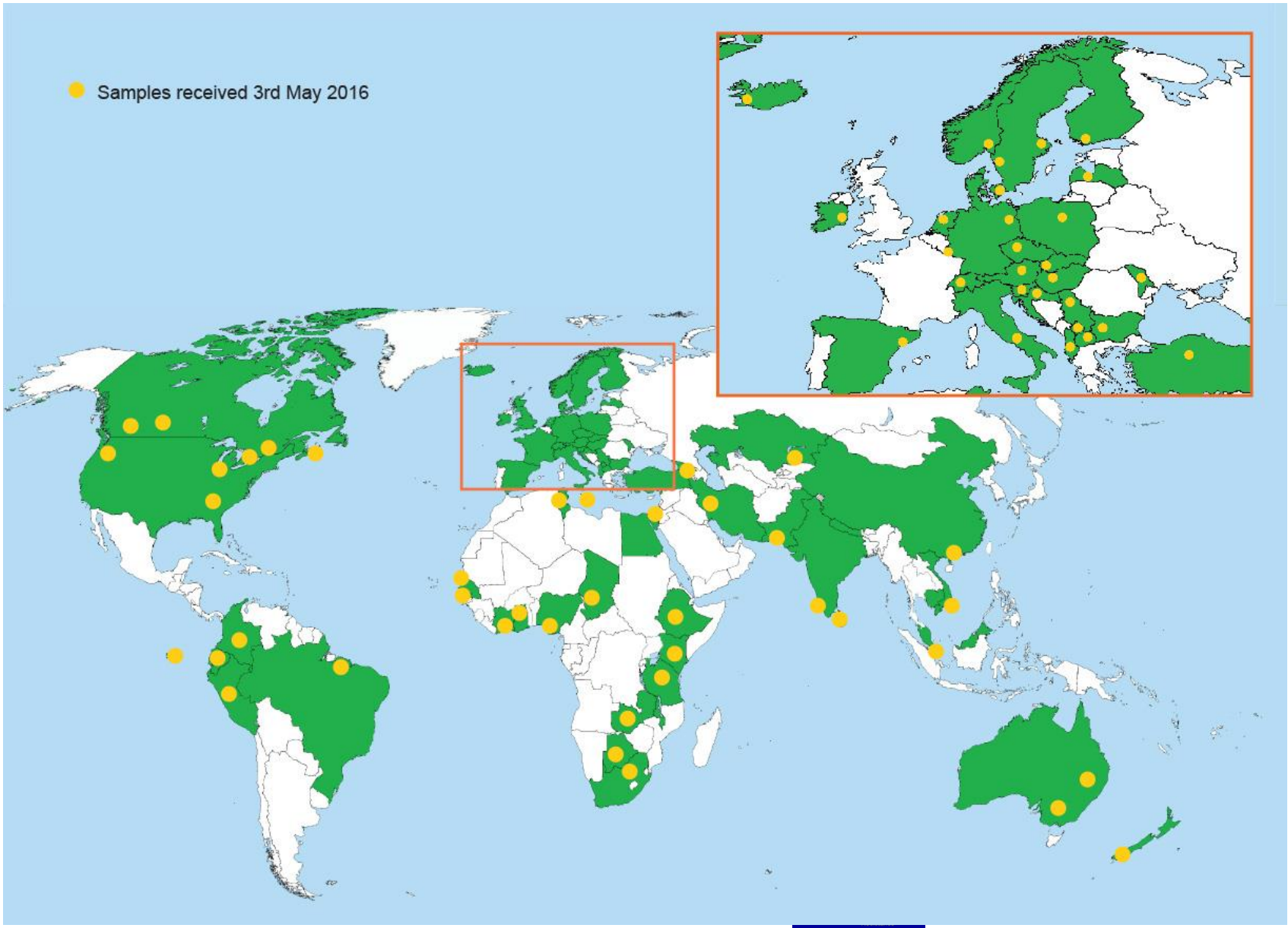




Disease hotspot surveillance - Slumcity of Kibera, Nairobi, Kenya

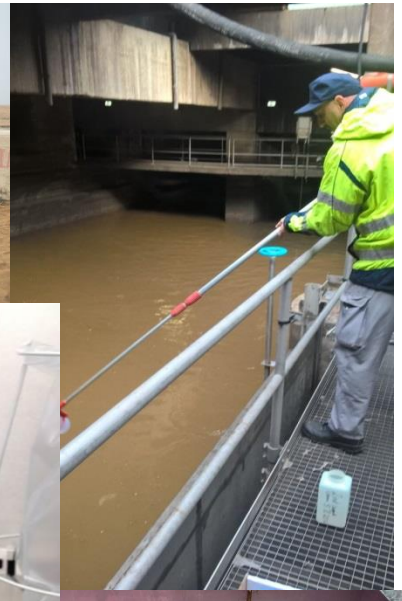
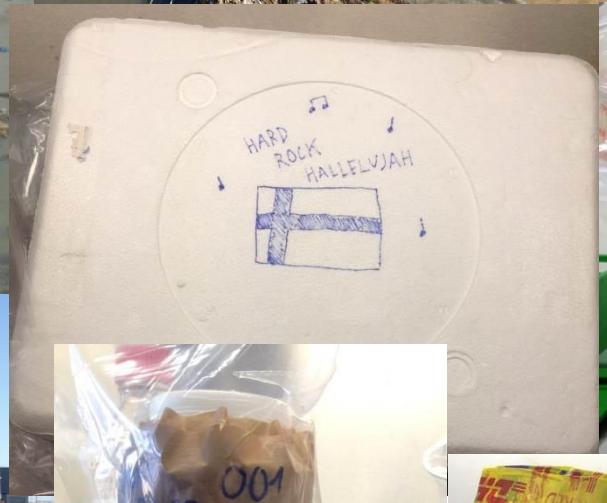
- Monitoring the vulnerable populations of Kibera
  - Collected 2 sewage samples every day for 3 months
- Demonstrate the application of using a metagenomics approach
  - to detect potential disease outbreaks
  - to develop corresponding intervention and prevention strategies
- Apply a temporal metagenomics analysis to identify and quantify human pathogens including bacteria and associated antimicrobial resistance, virus, and parasites
  - correlate with the disease trends from collected syndromic surveillance data and visits to the clinic
- Currently working with EBI to share data
  - PRJEB13833 - Kibera Sewage Project

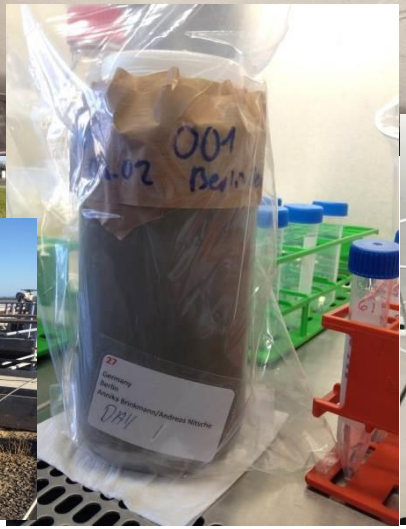
Global sewage surveillance - 2016



Global sewage surveillance - 2016





Global sewage surveillance - 2016

- Information about presence and distribution of (pathogenic) bacteria, virus and parasites on a global scale

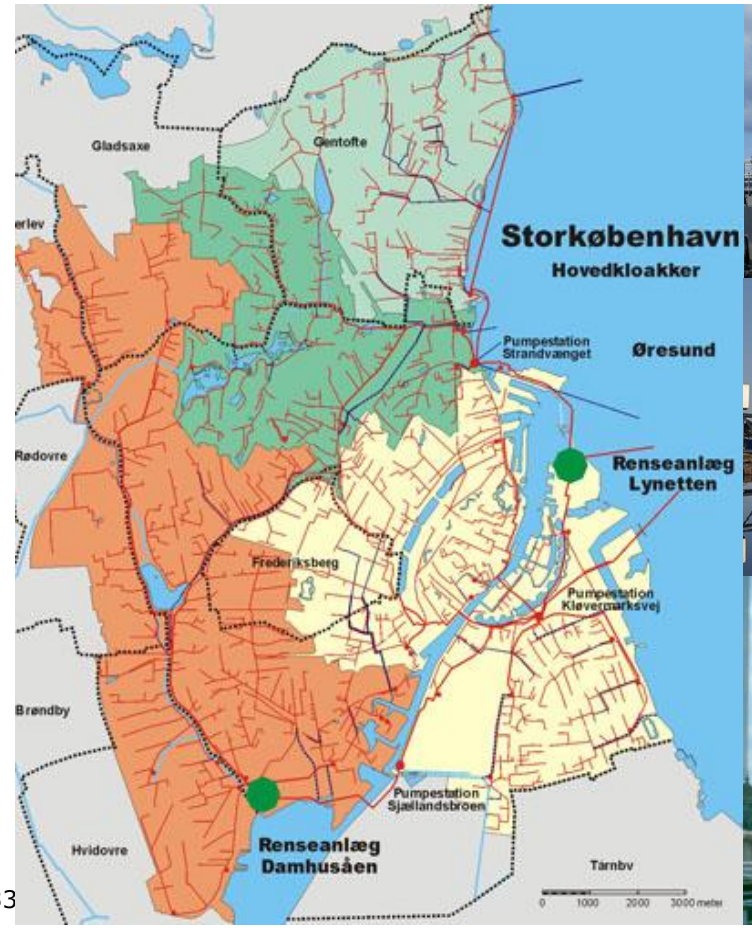
- A proof-of-concept of large-scale population surveillance using state-of-the-art technologies, metagenomics
  - Provide better and faster detection and control of health risks
  - Potentially reduce morbidity and mortality through rapid disease detection
  - Reduce development of antimicrobial resistance.
  - Improve treatment outcome and minimize disease spread

- Sample processing - Samples are divided into fractions
  - 250 ml for DNA (bacteria / virus / parasites) & RNA (virus)extraction
  - 250 ml for bacterial plasmid purification
  - 150 - 400 ml for Residue analysis

- PRJEB13831 - Global Sewage Project (Currently working with EBI to share data among COMPARE partners before release)

Copenhagen according to sewage - 2016

"Real time" sharing of data: PRJEB13832 - Copenhagen Sewage Project (public – instant release of data)



Copenhagen according to sewage - 2016

- Project start: 23-11-2015

- Samples are collected weekly - 80 samples till 02-05-2016
  - 3 sewage treatment plants:
    - Avedøre (12 samples)
    - Damhusåen (35 samples)
    - Lynetten (33 samples)

- Samples are picked up every two weeks and brought to DTU and processed within a week (turnaround time 3 weeks)
  - 250 ml for DNA (bacteria / virus / parasites) & RNA (virus)extraction
  - 250 ml for bacterial plasmid purification

- Sequenced in-house by MiSeq
  - The sequences are uploaded to EBI directly after sequencing

Thank you!!

From the EURL-AR team and our network