

## PARTICIPANT'S FILE

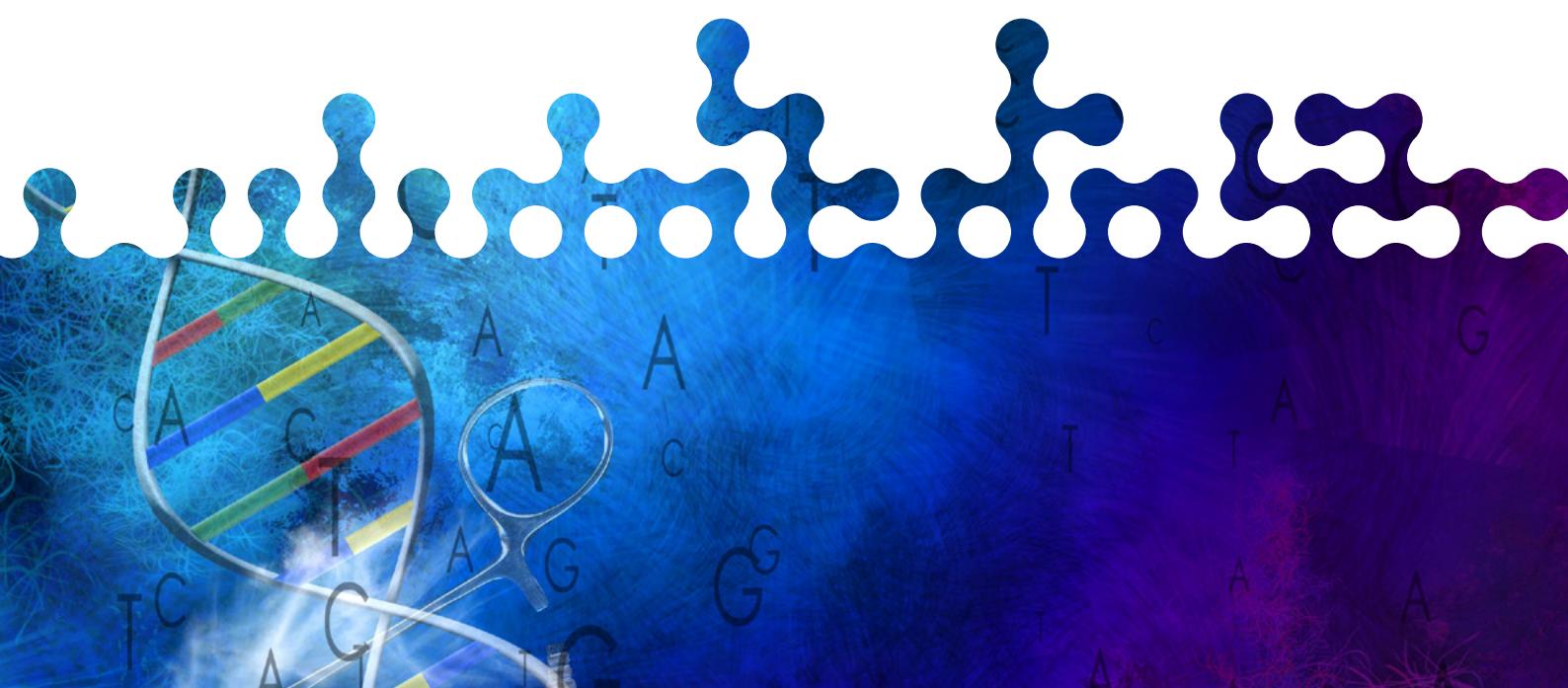
# Foodborne pathogens & whole genome sequencing: impact on public health protection

JOINT CONFERENCE

26-28 March 2019

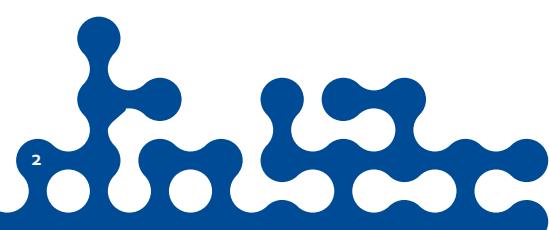
Maison de la RATP - Espace du Centenaire  
189 rue de Bercy  
75012 Paris - France

#FoodSafetyWGS2019



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It is a great pleasure to welcome you to Paris to attend the joint scientific conference co-organised by ANSES, BfR, DTU-Food and NIFDS on "Foodborne pathogens & whole genome sequencing (WGS): impact on public health protection".

Fuelled by the reduction in the cost of sequencing and the exceptionally rapid development of algorithmic and calculation resources, all the fields of biology are shaken by the genomic revolution. The food safety sector is no exception, and has seen in recent years its methods and approaches gradually shift into the genomic era. The OMIC revolution, however, is far from over and this meeting is an opportunity to share with a diverse audience of researchers, public health specialists, policy-makers and others, an overview of scientific breakthroughs and perspectives on the use, implementation and dissemination of WGS techniques in the public health area.

The easier access to full genome data offers scientists an unprecedented capacity to understand and predict many aspects of microbiological organisms: pathogen characterisation and typing, outbreak detection, risk assessment, antimicrobial resistance, pathogenicity, high-resolution epidemiology among others, are upgraded and revisited through the systematic use of WGS. Beyond knowledge on the biological properties of isolates, systematic genome sequencing enlarges our view of population structure, allows refinement of epidemiological models and dissemination patterns of microbial hazards. These aspects are crucial for food safety where the vision of pathogens at the population level is critical for proper anticipation and risk management and increasing traceability.

Pathogen genomics is not only changing the activities of laboratories involved in the investigation and management of infectious diseases but risk assessment is also questioned. This will be addressed during the conference by the presentations on developments in the field of modelling where genomic data feed new models for source attribution or risk assessment.

Beyond the scientific aspects, number of initiatives and publications clearly note the transformative potential of WGS to the delivery of microbiology and public health functions. WGS raises changes in the design, operation and workforce of public health agencies laboratories, in order to adopt this technology into routine practice. Organisational and management are the main questions to address with a focus onto the future of wet laboratory activities, or the recruitment and training of our technicians and scientists. It also stimulates reflection on the information technology (IT) infrastructures needed to adopt this technology into routine practice and take advantage of genomic data. Microbiologists should now integrate bioanalysis skills as statistics may play a bigger role in the area of microbiology than ever before, while still maintaining fundamental microbiological knowledge, complemented and not replaced by sequencing techniques. Less expensive, smaller and portable real-time devices for DNA and RNA sequencing offer a rapid dissemination of WGS techniques and this needs to be accompanied by a vision, including adequate financial support and scientific preparedness, in order to build the capacity required to master such techniques. This is one of the reasons why we are all gathered for this conference.

We are all confronted with the same questions and this joint conference, gathering our four institutions and numerous speakers from partners, and which will be attended by many scientists from other organisations, is a wonderful opportunity to exchange on our practices and our experience and find solutions that we can share. All these challenges will not be addressed unless we build strong scientific cooperation.

The expectations of civil society concerning food safety are immense, and we believe that the implementation of genomic approaches represents an effective new tool at our service for reducing the number of people who become ill due to foodborne infections. Food safety should nevertheless be supported by strengthened cooperation between the various actors in the food chain in order to reach its full potential. The promises of pathogens genomics require revisions of working practices and the implementation of new tools such as shared databases, common analytical methods and a clear understanding of scientific limitations by the various stakeholders. From this perspective, it is undeniable that the debate on data sharing is central and must be conducted taking into account all the interests at stake, to provide essential inputs to the development of political and practical strategies for improved pathogen data-sharing practices. To promote it, new regulatory actions may be necessary in the context of Nagoya agreement that requires further steps towards harmonized views and definitions requiring a close dialogue with all actors in the food chain.

This major scientific progress would not mean much if it was not used in a way that will benefit our ultimate goal: assess and manage risks in order to preserve public health and reduce the burden of infectious diseases in Humans induced by food-borne, animal-borne and water-borne pathogens. It will also be used to better protect animal and plant health in a "One Health" strategy.

For all these reasons, at the initiative of four national institutions for food safety and risk assessment in France, Denmark, Germany and South Korea, this conference brings together scientist, researchers, risk assessors and risk managers for an update on the impact on food safety that whole genome sequencing approaches already have, and will continue to have in the future.

This event is meant to be more than just lectures and posters: by making this event possible, we want to reaffirm our vision for science, with no borders in knowledge-sharing (and perhaps data-sharing) and looks forward to the future thanks to a living and fruitful cooperation at the European and international level. ■

**Roger GENET**  
Director General, ANSES, France

**Andreas HENSEL**  
President, BfR, Germany

**Christine NELLEMANN**  
Director, National Food Institute,  
DTU, Denmark

**Kyung Won SEO**  
Director General of Drug Evaluation  
Department, Acting Director  
General, NIFDS, South Korea



# FOODBORNE PATHOGENS & WHOLE GENOME SEQUENCING

## JOINT CONFERENCE

### TUESDAY 26 MARCH

13:30	Registration
14:00	<b>Opening</b> <b>Welcome and opening remarks from ANSES</b> Roger GENET, ANSES, France <b>Opening remarks from DTU</b> Christine NELLEMANN, National Food Institute, DTU, Denmark <b>Opening remarks from BfR</b> Andreas HENSEL, BfR, Germany <b>Opening remarks from NIFDS</b> Hyo-Sun KWAK, NIFDS, South Korea
14:50	<b>Introductory Keynote speech</b> <b>The Promise of Microbial Genomics: How Microbiology is Standing Up to the Many Challenges of a 21<sup>st</sup> Century Food Supply</b> Eric BROWN, Center for Food Safety and Applied Nutrition (FDA), USA
15:50	Coffee break
16:20	<b>SESSION 1</b> <b>WGS for microbiological surveillance and epidemiology</b> <b>CHAIRS:</b> Jørgen SCHLUNDT, Nanyang Technological University (NTU), Singapore and Hyo-Sun KWAK, National Institute of Food and Drug Safety Evaluation (NIFDS), Ministry of Food and Drug Safety, South Korea <b>KEYNOTE SPEAKER:</b> <b>Genomic insights into the epidemiology and surveillance of <i>Vibrio cholerae O1</i> infections</b> François-Xavier WEILL, Institut Pasteur, France  <b>Surveillance of <i>Listeria monocytogenes</i> in foodstuffs and food processing plants by WGS in Germany</b> Stefanie LÜTH, Federal Institute for Risk Assessment, Freie Universität Berlin, Germany  <b>Rapid Detection and Identification of Foodborne Pathogens using Single Nucleotide Polymorphism (SNP) Profiling of Their Whole Genome Sequences</b> Ju-Hoon LEE, National Institute of Food and Drug Safety Evaluation (NIFDS), Kyung Hee University, South Korea  <b>Past, present and future in the Danish antimicrobial resistance monitoring programme (DANMAP)</b> Valeria BORTOLAI, National Food Institute, DTU, Denmark
18:05	End of the day



## WEDNESDAY 27 MARCH

8:30 Registration

### SESSION 2

#### Genomics for foodborne pathogens characterization and outbreak investigation

**CHAIRS:** **Mark ALLARD**, Food and Drug Administration (FDA), Office of Regulatory Science, Division of Microbiology, USA and **Burkhard MALORNY**, Federal Institute for Risk Assessment (BfR), Germany

**KEYNOTE SPEAKER:** **Impact of whole genome sequencing on the investigation of foodborne outbreaks of Shiga toxin-producing Escherichia coli serogroup O157:H7**  
**Claire JENKINS**, Public Health England, United Kingdom

**Building capacity in WGS analysis for detection of deliberate outbreaks**  
**Jette Sejer KJELDGAARD**, National Food Institute, DTU, Denmark

**Genomic characterization of *Clostridium perfringens* strains involved in food poisoning outbreaks in France 2013-2017**

**Abakabir Mahamat ABDELRAHIM**, ANSES, France

**Foodborne Outbreaks Investigation and Whole Genome Sequences Analysis In Korea MFDS**  
**Jin-Hee HWANG**, National Institute of Food and Drug Safety Evaluation (NIFDS), Ministry of Food and Drug Safety, South Korea

10:45 Coffee break

### SESSION 3

#### Quantitative risk assessment modelisation and source attribution in the genomic era

**CHAIRS:** **Mirko ROSSI**, European Food Safety Authority (EFSA), Italy and **Gilles SALVAT**, ANSES, France

**KEYNOTE SPEAKER:** **Towards 'next-generation' source attribution and microbial risk assessment of foodborne pathogens**  
**Lapo MUGHINI-GRAS**, RIVM, the Netherlands

**The use of Whole Genome Sequencing in risk assessment: Application to the cold smoked salmon-related listeriosis risk model**

**Laurent GUILLLIER**, ANSES, Food Safety Laboratory, Unit Salmonella and Listeria, France

**Campylobacter: recent knowledge using genomics and metagenomics**

**Marianne CHEMALY**, ANSES, Laboratory of Ploufragan-Plouzané-Niort, Unit of Hygiene and Quality of Poultry and Pork Products, France

**RAKIP: Resources for harmonized annotation and efficient exchange of risk assessment models**  
**Matthias FILTER**, Federal Institute for Risk Assessment (BfR), Germany

13:00 Free time for lunch



# FOODBORNE PATHOGENS & WHOLE GENOME SEQUENCING

## JOINT CONFERENCE

### WEDNESDAY 27 MARCH

14:30

#### SESSION 4

##### Pipelines and workflows for WGS data analysis

**CHAIRS:** Soohwan SUH, National Institute of Food and Drug Safety Evaluation (NIFDS), Ministry of Food and Drug Safety, South Korea and Eric BROWN, Center for Food Safety and Applied Nutrition, Food and Drug Administration, USA

**KEYNOTE SPEAKER: Integration of genomics in outbreak detection and investigation of food-borne pathogens: state-of-the-art and challenges**

Mirko ROSSI, European Food Safety Authority (EFSA), Italy

**PathoLive - Pathogen detection while sequencing**

Simon TAUSCH, Federal Institute for Risk Assessment (BfR), Germany

**Adaptation to animal sources of *Salmonella enterica* subsp. *enterica* deciphered by Genome Wide Association Study and Gene Ontology Enrichment Analysis at the pangenomic scale**

Nicolas RADOMSKI, ANSES, Food Safety Laboratory, Genome Analysis Modelling Risk (GAMeR), France

**First steps towards incorporation of whole-genome sequencing data in exposure assessment: Machine Learning and Network-Diffusion approaches**

Pimlapas LEEKITCHAROENPHON, Technical University of Denmark, National Food Institute, DTU, Denmark

16:15

#### Coffee break

16:45

#### SESSION 5

##### Antimicrobial resistance

**CHAIRS:** Jean-Yves MADEC, ANSES, France and Valeria BORTOLAIA, Technical University of Denmark, National Food Institute, DTU, Denmark

**KEYNOTE SPEAKER: The presence and future in antimicrobial resistance surveillance**

Rene Sjøgren HENDRIKSEN, Technical University of Denmark, National Food Institute, DTU, Denmark

**Plasmid-mediated colistin resistance in German *Salmonella enterica* strains isolated from livestock, food and the environment**

Maria BOROWIAK, Federal Institute for Risk Assessment (BfR), Germany

**One Health Approach on AMR surveillance in Korea**

Soohwan SUH, National Institute of Food and Drug Safety Evaluation (NIFDS), Ministry of Food and Drug Safety, South Korea

**WGS and antimicrobial resistance**

Isabelle KEMPF, ANSES, Ploufragan-Plouzané-Niort Laboratory, France

18:15

#### Cocktail (until 20:00)



## THURSDAY 28 MARCH

8:30

Registration

9:00

### SESSION 6

#### Metagenomics for food safety

**CHAIRS:** **Carlus DENEKE**, Federal Institute for Risk Assessment (BfR), Germany  
and **Michel-Yves MISTOU**, ANSES, France

**KEYNOTE SPEAKER:** **Challenges and opportunities in foodborne virus genomics**  
**Marion KOOPMANS**, Erasmus Medical Center, The Netherlands

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##### **Application of metagenomics for the detection of foodborne pathogens**

**Josephine GRUETZKE**, Federal Institute for Risk Assessment (BfR), Germany

##### **Identification of Probiotic Bacteria in Foods through Metagenomic Approach**

**Woojung LEE**, National Institute of Food and Drug Safety Evaluation (NIFDS), Ministry of Food and Drug Safety, South Korea

##### **Global surveillance of antimicrobial resistance through global sewage**

**Pimplapas LEEKITCHAROENPHON**, Technical University of Denmark, National Food Institute, DTU, Denmark

10:45

Coffee break

11:00

### ROUND TABLE 1

#### Handling multi countries foodborne outbreaks

**MODERATORS:** **Charlotte GRASTILLEUR**, ANSES, France and **Hyo-Sun KWAK**, National Institute of Food and Drug Safety Evaluation (NIFDS), Ministry of Food and Drug Safety, South Korea

**Stefano MORABITO**, Istituto Superiore di Sanità (ISS), Italy

**Martial PLANTADY**, Health Food Safety Directorate-General (DG SANTE), European Commission, Belgium

**Johanna TAKKINEN**, European Centre for Disease Prevention and Control (ECDC), Sweden

**Henriette de VALK**, Santé publique France, France

11:45

### ROUND TABLE 2

#### Data sharing and regulatory issues in the WGS era

**MODERATORS:** **Rene Sjøgren HENDRIKSEN**, National Food Institute, DTU, Denmark  
and **Karsten NÖCKLER**, Federal Institute for Risk Assessment (BfR), Germany

**Marc ALLARD**, Food and Drug Administration (FDA), Office of Regulatory Science, Division of Microbiology, USA

**George HARINGHUIZEN**, National Institute for Public health and the Environment (RIVM), The Netherlands

**Mirko ROSSI**, European Food Safety Authority (EFSA), Italy

**Jørgen SCHLUNDT**, Nanyang Technological University (NTU), Singapore

12:30

### Closing speech

**Roger GENET**, ANSES, France

13:00

End of the Joint scientific Conference

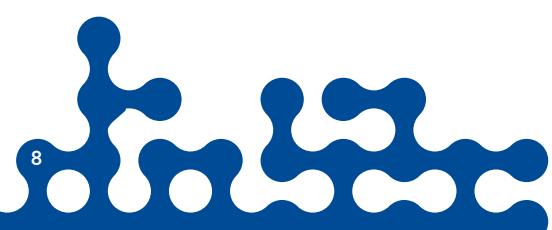


TUESDAY 26 MARCH

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# Introductory Keynote speech

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## Eric BROWN / Center for Food Safety and Applied Nutrition (FDA), USA

Dr. Eric W. Brown has been with the Food and Drug Administration's Center for Food Safety and Applied Nutrition (CFSAN) since 1999 and currently serves as Director of the Division of Microbiology in the Office of Regulatory Science. Here, he oversees a group of 60 food safety microbiology researchers and support scientists engaged in a multi-parameter research program to develop and apply microbiological and molecular genetic strategies for detecting, identifying, and differentiating bacterial foodborne pathogens such as *Salmonella*, *Listeria*, and shiga-toxin producing *E. coli*. Recently, his laboratory has been instrumental in adapting next-generation sequencing technologies to augment foodborne outbreak investigations and to ensure preventive control and compliance standards at the FDA including the establishment of the GenomeTrakr whole-genome sequencing network for food safety. Dr. Brown received his M.Sc. in Microbiology from the National Cancer Institute/Hood College joint program in the biomedical sciences in 1993 and his Ph.D. in Microbial Genetics from The Department of Biological Sciences at The George Washington University in 1997. He has conducted research in microbial evolution and genetics as a research fellow at the National Institutes of Health, the U.S. Department of Agriculture, and as an Assistant Professor of Microbiology at Loyola University of Chicago. He has been a member of the American Society for Microbiology since 1994 and was inducted as a Fellow of the American Academy of Microbiology in 2015. He has co-authored more than 200 refereed publications and book chapters on the molecular differentiation, evolutionary genetics, and ecological persistence of bacterial pathogens.

## The Promise of Microbial Genomics: How Microbiology is Standing Up to the Many Challenges of a 21<sup>st</sup> Century Food Supply

High-resolution forensic tools are essential for aiding in tracing foodborne contamination events back to their source, and in this regard, whole genome sequencing (WGS) is rapidly transforming microbiological subtyping in the food safety laboratory. When coupled with powerful bioinformatic pipelines, accurate and stable genetic changes can be identified across pathogen genomes that can distinguish strains to the source level including individual farms or facilities and specific geographic locales.

This is true even among highly homogeneous strain populations such as *Salmonella* Enteritidis and other salmonellae that have remained largely recalcitrant to differentiation by conventional typing approaches. Numerous published examples illustrate the ability of WGS to discern genetic relatedness of otherwise indistinguishable isolates and point to WGS as an important tool in the traceability of contamination events. To this end, FDA has created an integrated pilot network of state and federal laboratories to use whole genome sequencing to enhance traceback of foodborne pathogens. Known as GenomeTrakr and now comprising several government food safety agencies as well as nearly three-dozen state, academic, and international partners, the network is creating a publically available, global database containing the genetic makeup of tens of thousands of foodborne disease-causing bacteria including *Salmonella*.

At present, WGS impacts regulatory science in FDA's Foods Program in several ways including: (i) support of traceability efforts during foodborne contamination events; (ii) enhanced regulatory casework for high-risk commodities and compliance standards; and (iii) quality assurance of food microbiological sampling programs. Taken together, regular applications of WGS underscore its extraordinary utility in food safety as well as the potential for complete characterization of bacterial pathogens as they emerge in the food supply.



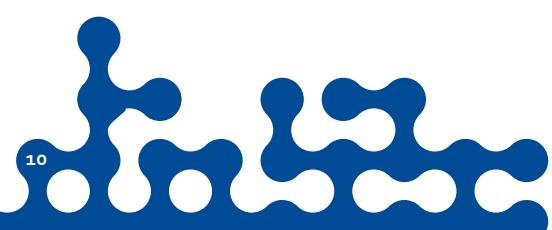
TUESDAY 26 MARCH

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# SESSION 1

## WGS for microbiological surveillance and epidemiology

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## CHAIRS

### Jørgen SCHLUNDT / Nanyang Technological University (NTU), Singapore

*Professor, Food Science at NTU. JS has worked nationally and internationally in research-based regulatory food safety 1983-99. Later Director Food Safety at the World Health Organization and Director National Food Institute of Denmark. JS participated in international development of food safety Risk Analysis and the initiation of the Global Microbial Identifier, suggesting a global database of Whole Genome Sequences of all microorganisms.*

### Hyo-Sun KWAK / National Institute of Food and Drug Safety Evaluation (NIFDS), Ministry of Food and Drug Safety, South Korea

*Dr. Kwak as a research microbiologist, she has been working for Korea Ministry of Food and Drug Safety(MFDS) for 27 years. She has been working in the area of risk assessment, methodology development, food standard establishment and act against foodborne disease outbreak in MFDS. She joined US Centers for Disease Control and Prevention(CDC) as a visiting scientist in 1995, and also Korea CDC for 1 year according to personnel interchange program in Korea.*

*Dr. KWAK launched the 'National Antimicrobial Resistance Management Program' in Korea in 2003, and continuously contributed to the reduction of antimicrobial resistance and use in Korea. In 2007, she worked actively for designating Korea as a hosting country of the Codex Task Force on antimicrobial resistance (AMR) and served as a secretariat of Codex TF AMR for 4 successful years. She also served as WHO expert group (AGISAR, Advisory Group of Integrated Surveillance of Antimicrobial Resistance) from 2014 to 2018, and participate in the ADB food safety management project from 2013 to 2017 in order to upgrade of the food safety level of LAO PDR. For the purpose of rapid reacting of foodborne outbreaks, she launched the MFDS mobile lab system, identifying disease causing agents within 4 hrs, in Korea for on-site outbreak investigation. This mobile lab has been used as a useful tool for prevention and control of foodborne diseases in international events such as 2018 Pyeongchang Winter Olympic games.*

*As a director of Division of Food Microbiology , she leaded the research group, 'Using NGS in foodborne pathogen management' since 2014 to build up genome DB of foodborne pathogens as well as strain bank for microbial pathogens isolated from outbreak in Korea. She also involved in research developing high-throughput data analysis software such as SNP pipeline, HGTree and Probiotics-analysis pipeline. Dr. Kwak is enthusiastic in building national DB for foodborne pathogens as well as improving quality of public health.*



# FOODBORNE PATHOGENS & WHOLE GENOME SEQUENCING

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## KEYNOTE SPEAKER

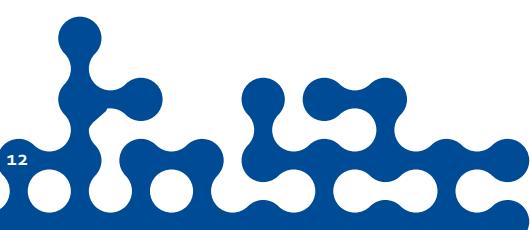
François-Xavier WEILL / Institut Pasteur, Paris, France

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François-Xavier Weill, MD, PhD is Associate Professor at the Pasteur Institute, Paris, France. For the last 10 years, he has been heading the Enteric Bacterial Pathogens Unit. His research interests are the population structure and transmission dynamics of emerging, epidemic, and antimicrobial drug resistant enteric bacterial pathogens, as well as molecular and genomic epidemiology. He has published over 150 research papers in international journals, including *Science*, *Nature*, and *Nature Microbiology*.

## Genomic insights into the epidemiology and surveillance of *Vibrio cholerae* O1 infections

Cholera is an acute intestinal infection that leads to a rapid and severe dehydrating diarrhea, and is caused by *Vibrio cholerae* O1 and O139 producing cholera toxin. Aside from being a prominent human pathogen, exploratory analyses have demonstrated since the 1970s that *V. cholerae* is an integral member of many coastal, estuarine, and brackish water ecosystems, as are other *Vibrio species*, in which it is often associated with copepods and zooplankton. However, confusion surrounded the relationships between globally circulating pandemic *Vibrio cholerae* clones and local bacterial populations. Data from whole genomes are now providing fresh insights into the epidemiology and surveillance of *Vibrio cholerae* O1 infections. ■



## Stefanie LÜTH / Federal Institute for Risk Assessment, Freie Universität Berlin, Germany

2016: Master of Science, Molecular Life Science, Universität zu Lübeck

Since 2017: Doctoral candidate at the German Federal Institute for Risk Assessment (BfR)

### Surveillance of *Listeria monocytogenes* in foodstuffs and food processing plants by WGS in Germany

Sylvia Kleta / Federal Institute for Risk Assessment, Germany ; Sven Halbedel / Robert Koch-Institute, Germany ; Antje Flieger / Robert Koch-Instituten, Germany ; Sascha Al Dahouk / Federal Institute for Risk Assessment, RWTH Aachen University, Germany

Listeriosis, caused by the bacterium *Listeria monocytogenes* (*Lm*), has the highest proportion of hospitalised cases of all foodborne zoonoses under EU surveillance. Human infections mainly occur in elderly, pregnant and immunocompromised people after consumption of contaminated foodstuffs. Its high case fatality rate renders listeriosis a major public health concern. Worryingly, in Germany, case numbers of human listeriosis have been steadily increasing for several years. For effective surveillance and disease control, comprehensive molecular typing of *Lm* isolated from food, food-processing plants and humans is indispensable. Still, the analytical basis for that purpose needs to be improved by using the potential of novel technologies.

Since 2016, in-house whole genome sequencing (WGS) is becoming established as a typing method for *Lm* isolates from food and food-processing plants at the German National Reference Laboratory at BfR. Currently, WGS is the method of choice for event-driven investigations. In parallel, within the scope of the research project MolTypList<sup>1</sup>, representative typing data are retrospectively generated along with the validation and establishment of methodical standards for WGS as a typing tool.

On the one hand, our WGS-database provides important insights into the population structure of *Lm* in the food chain in Germany and forms the basis for an improved risk assessment. Within various WGS-based clusters, a very close genetic relationship between *Lm*-isolates from food products sampled on different stages of the product's life span (manufacturer, distributor) or between isolates from food product and food-processing plant could be proven. This reflects the way of *Lm*-contamination in food production and retail systems. Furthermore, genetic relatedness of isolates sampled at different time points possibly gives a hint to persistence of specific genotypes in food-processing plants, thus highlighting the need for improved hygiene measures.

On the other hand, our WGS-database can be used for accelerated and more accurate trace-back of human infections to contaminated foodstuffs to resolve outbreaks. In this way, WGS-typing results provided the key to tracing back several listeriosis clusters in Germany to contaminated food products and/or responsible manufacturers and to eliminating the source of infection. These listeriosis clusters also included long-lasting outbreaks over several years associated with a large number of cases. Altogether, WGS-based typing has proven to be a valuable tool in outbreak investigations in Germany and will make a decisive contribution to reduce the burden of listeriosis. ■

<sup>1</sup> -This project is supported by a grant of the Federal Ministry of Health (GE20160326) within the framework of the German Research Platform for Zoonoses.



Ju-Hoon LEE / National Institute of Food and Drug Safety Evaluation (NIFDS), Kyung Hee University, South Korea

Dr. Ju-Hoon Lee received B.S. and M.S. degrees from the Department of Food Science and Technology at Seoul National University, South Korea, and a Ph.D. from the Department of Food Science and Nutrition at the University of Minnesota, USA. For Postdoc training, he was a research associate at Cargill Genomics Institute, USA. Since 2011, he has worked as an assistant and associate professor in the Department of Food Science and Biotechnology, Kyung Hee University. Dr. Lee's major research interest involves microbial genomics and bioinformatics for comparative and functional analysis of probiotics and food-borne pathogens to further understand their evolution, physiology, and ecology as well as their interactions with foods and other food microbes.

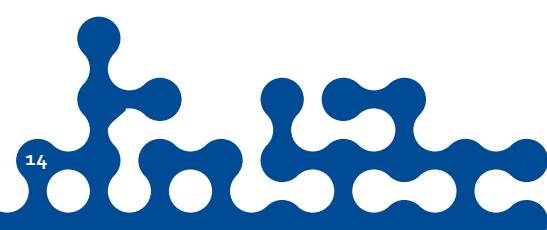
## Rapid Detection and Identification of Foodborne Pathogens using Single Nucleotide Polymerism (SNP) Profiling of Their Whole Genome Sequences

Rapid detection and identification of specific pathogen in food samples are very important to prevent food-borne outbreak and its propagation to the public as well as to confirm the epidemiologic history. While culture-dependent detection method using selective media and DNA-based detection method including Rep-PCR and PFGE have been widely used, these methods have many limitations such as long culturing time, no detection of unculturable pathogens, and low accuracy and fidelity due to short DNA sequence-based detection and identification.

These days, next-generation sequencing (NGS) is very popular and cheap, according to the development of new NGS sequencing technologies. Therefore, NGS has been very useful tool to explore whole genome sequences (WGS) of food-borne pathogens to enhance the accuracy and fidelity for their rapid detection and identification. In 2014, Center for Food Safety and Applied Nutrition (CFSAN) of US FDA developed a SNP pipeline program to detect and identify specific food-borne pathogens using Illumina MiSeq sequencing-based single nucleotide polymorphism (SNP) patterns to improve the accuracy of PulseNet based on PFGE band patterns. In this study, FORC SNPing pipeline program was developed to improve the analysis speed and accuracy of CFSAN pipeline program.

This updated program consists of five steps such as data quality control using Trimmomatic, alignment to reference sequence using BOWTIE, data manipulation using SAMtools/Picard, variants calling from multiple files using Python/GATK, and generation of result files using FastTree. To validate this new program, FORC SNPing was compared to CFSAN SNP pipeline, showing higher sensitivity (99.0% over 98.7%) with the same accuracy and specificity, suggesting high analysis depth and accuracy. In addition, to reduce the analysis time using FORC SNPing, all calculation was changed from serial to parallel process. Using this parallel calculation method, total analysis time for 100 and 348 samples reduced to 26% (2 h 49 min over 3 h 50 min) and 30% (14 h 58 min over 21 h 22 min), respectively. Therefore, it is clearly understood that updated FORC SNPing pipeline program can make rapid detection and accurate identification of specific food-borne pathogen possible.

However, to maximize the accuracy of rapid detection and identification as well as the usability for confirmation of epidemiologic history of the specific food-borne pathogens, database should be extended with more WGS results as well as more accurate reference genome sequences of food-borne pathogens in the world. Furthermore, FORC SNPing pipeline program needs to be optimized and updated with this extended database. ■



## Valeria BORTOLAIA / National Food Institute, DTU, Denmark

*Dr. Valeria Bortolaia is a Senior Researcher at the Technical University of Denmark where she works in the field of antibiotic resistance at the human-animal health interface. Her scientific goal is to limit spread of antibiotic resistance thus preserving our ability to treat infectious diseases. Dr. Bortolaia's research targets the dynamics of resistance transfer among bacteria from animals, food and humans and the development of genomic-based tools for rapid detection of antibiotic resistance.*

### Past, present and future in the Danish antimicrobial resistance monitoring programme (DANMAP)

The Danish Integrated Antimicrobial Resistance Monitoring and Research Programme (DANMAP) was established in 1995 as a pioneering initiative aiming to i) monitor the occurrence of antimicrobial resistance (AMR) in bacteria from food animals, food and humans, ii) monitor antimicrobial use (AMU) in humans and in animals, iii) record trends in AMU and AMR and iv) demonstrate associations between AMU and AMR occurrence. Since its inception, DANMAP proved to be a valuable instrument driving important changes in AMU such as the ban of use of growth promoters and the establishment of thresholds for AMU in food animals, among others. DANMAP has also contributed significantly to promote research into AMR mechanisms and transmission with the overall goal to promote public health.

While the main objectives of DANMAP remained unchanged over time, sampling schemes and analytical methods have adapted to reflect advances in scientific and technological knowledge. This ensures that data generated are always of the highest standard and are comparable with contemporary data from other countries, but also limits the comparability of results collected over time.

All these aspects along with their impact on clinical and veterinary practice and on public health will be discussed at the conference. In addition, insights into the ongoing research to evaluate the applicability of whole genome sequencing (WGS) and possibly of metagenomics for AMR monitoring in the DANMAP context will be presented. ■



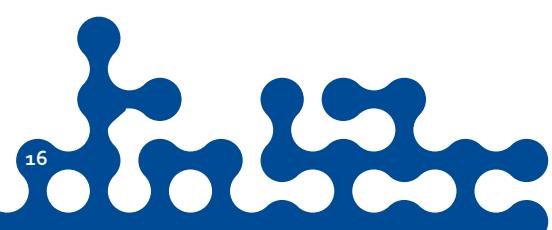
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## SESSION 2

# Genomics for foodborne pathogens characterization and outbreak investigation

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## CHAIRS

**Marc ALLARD** / Food and Drug Administration (FDA), Office of Regulatory Science,  
Division of Microbiology, USA

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*Marc W. Allard is a Senior Biomedical Research Services Officer in the Division of Microbiology in FDA's Office of Regulatory Science. Dr. Allard joined The US FDA in 2008 where he uses Whole Genome Sequencing (WGS) of foodborne pathogens to identify and characterize outbreaks of bacterial strains, particularly *Salmonella*, *E. coli*, and *Listeria*. Dr. Allard specializes in both phylogenetic analysis, as well as the biochemical laboratory methods which generate the WGS information. Dr. Allard helped develop the first distributed network of laboratories that utilize whole genome sequencing for pathogen identification and traceback called the GenomeTrkr database, which is part of the NCBI Pathogen Detection web site. These tools are used daily for outbreak investigations. Dr. Allard acts as senior scientist to advise the US FDA on both WGS and phylogenetic methods as they apply to public health.*

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**Burkhard MALORNY** / Federal Institute for Risk Assessment (BfR), Germany

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*Burkhard Malorny is a genome food microbiologist at the German Federal Institute for Risk Assessment. He leads provisionally the Study Centre for Genome Sequencing and Analysis. The Centre provides a platform for whole genome sequencing bacterial isolates and viruses as well as bioinformatic analysis tools as service for the microbial National Reference Laboratories located at BfR. He is author of approximately 100 peer reviewed publications and book chapters. He is member of several national and international working groups dealing with the standardization and application of whole genome sequencing in food microbiology.*



# FOODBORNE PATHOGENS & WHOLE GENOME SEQUENCING

JOINT CONFERENCE

## KEYNOTE SPEAKER

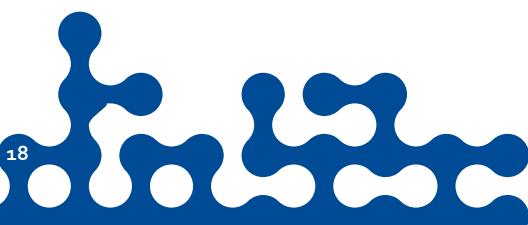
**Claire JENKINS** /Public Health England, United Kingdom

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*I started working for Public Health England, formerly the Health Protection Agency, as a Clinical Scientist in 1996. I became head of the E. coli Reference Laboratory in 2012 and deputy head of the Gastrointestinal Bacteria Reference Unit in 2014, the same year as we implemented whole genome sequencing as our routine typing method for surveillance and outbreak investigation.*

## Impact of whole genome sequencing on the investigation of foodborne outbreaks of Shiga toxin-producing *Escherichia coli* serogroup O157:H7

During outbreak investigations caused by gastrointestinal bacterial foodborne pathogens, whole genome sequencing (WGS) analysis provides an unprecedented level of strain discrimination and a robust case definition for case ascertainment. WGS data also enables us to explore the evolutionary context of outbreak strains. At Public Health England, a standardised enhanced surveillance questionnaire (ESQ) for every case of Shiga Toxin-producing *Escherichia coli* (STEC) is linked to every STEC genome sequence. The ESQ provides information on clinical symptoms, travel history, animal and environmental exposures and food history. The epidemiological information linked to sporadic cases within the outbreak cluster may shed light on likely transmission routes and/or the geographical origin of outbreak associated strains. This approach has been applied to recent (and historic) foodborne outbreaks, and the impact on the public health investigation has been evaluated.



## Jette Sejer KJELDGAARD / National Food Institute, DTU, Denmark

*Being a food scientist with a genuine passion for microbiology, I have a wide background in food safety, zoonoses, WGS and antimicrobial resistance. My work focus is on international activities within the area of antimicrobial resistance and genomic epidemiology, and I take active part in the Danish food- and waterborne outbreak investigation based on WGS analysis.*

### Building capacity in WGS analysis for detection of deliberate outbreaks

Genomic analyses include powerful tools for characterization of pathogens and outbreak detection increasingly used for these purposes worldwide. In the past year, DTU Food have been organising proficiency tests to evaluate laboratory capacities for performing these analyses related to identification of biological threats and deliberately released outbreaks, in coordination with the United Nations Office of Disarmament Affairs (UNODA) and the Swedish Defence Research Agency (FOI), funded by the United States Department of State.

The proficiency tests (PTs) aimed to strengthen the ability to detect a biological threat based on genomic or metagenomic analyses of pathogens or food and water samples. The PTs has been open for laboratories from different sectors, including public health-, veterinary-, general microbiological- and biodefence-associated laboratories with a wide global distribution in the networks of COMPARE, GMI and UNSGM (United Nations Secretary-General's Mechanism for Investigation of Alleged Use of Chemical and Biological Weapons). When an allegation is made of an intentional cause for a biological threat or disease outbreak, an investigation for verification purposes needs to discriminate between a deliberate release of a harmful agent and the natural background. This requires a detailed analysis of the identified agent. The ability of the participants to correctly detect and report the deliberately released bacterium is thus essential for both implementing rapid and appropriate control measures but also to avoid unnecessary fear in the population due to false threats.

This presentation will describe how whole genome sequencing of pathogens has been applied to evaluate laboratories' capabilities of performing, analysing, evaluating and reporting reliable genomic data and/or bioinformatics analysis of biological incidences. The series of three PTs were designed for covering analyses of increasing genomics abilities on simulated datasets. This started with characterisation of pathogens and possibly altered virulence genes (PT<sub>1</sub>, early 2018), moving into identification of pathogens in whole community sequencing datasets (metagenomic data) of food and water (PT<sub>2</sub>, late 2018). The last exercise (PT<sub>3</sub>) is still ongoing and covers phylogenetic analysis of single genomes for identification of a deliberately released disease outbreak, combined with a forensic approach to trace the incriminating food source connected to the disease outbreak.

The PTs have highlighted some areas of analysis that requires special attention related to bioinformatics analysis of biological incidences. The presentation will briefly cover the setup of the PTs and the overall results related to the evaluation of laboratory capacities, and elucidate some of the benefits and drawbacks in measuring these capacities based on genomic analysis. ■



## Abakabir Mahamat ABDELRAHIM / ANSES, France

Abakabir Mahamat Abdelrahim, PhD (2019) is a research project leader in the SBCL (Staphylococcus, Bacillus and Clostridium) unit at Anses. For the past 4 years, as part of his thesis, he has been developing molecular tools for the identification and characterization of *Clostridium perfringens* strains implicated in FPO in order to understand the occurrence of toxic episodes. He is currently continuing his work on the characterization of *C. perfringens* strains in the European postdoctoral project ToxDetect at ANSES.

### Genomic characterization of *Clostridium perfringens* strains involved in food poisoning outbreaks in France 2013-2017

*Clostridium perfringens* represents the fourth agent responsible for food poisoning outbreaks (FPO) in France and the rest of Europe. The bacterium is classified into seven toxinotypes (A-G) based upon the production of six major toxins, namely CPA, CPB, ETX, ITX, CPE and NetB. It has been demonstrated that the enterotoxin CPE, encoded by the *cpe* gene, is essential to the development of gastroenteritis. However, twenty-two virulence factors comprising toxins and hydrolytic enzymes produced by *C. perfringens* strains are currently established in the literature for which no functional data are available, and their role in FPO remains unknown since the reference method namely -EN NF ISO 7937- used to certify the presence of *C. perfringens* involved in FBO is not able to provide any phenotypic information with regard to their virulence. In addition, no genotypic characterization is currently available to link *C. perfringens* strains to a particular reservoir or their prevalence in FPO.

In order to improve the characterization of *Clostridium* strains involved in FPO, we first studied the virulence gene profile of *C. perfringens* strains considering all *C. perfringens* virulence factors identified to date. The result revealed a considerable diversity, since 15 different virulence gene profiles were obtained from a collection of 146 strains implicated in 44 FBOs in the Paris region between 2013 and 2018. Heterogeneous virulence gene profiles from multiple strains isolated during the same FPO have been demonstrated in particular for the presence of the *cpe* gene. The results of virulence gene profiling also revealed cases in which *C. perfringens* was the only pathogen identified in the incriminated food, but none of the recovered strains was positive for the *cpe* gene, which brings into hypothesis that *cpe-* strains may be associated with FPO. Then, a genomic analysis was performed in order to identify genetic markers that characterize *C. perfringens* strains involved in FPO. Phylogenomic reconstruction using core genome SNPs identified two distinct clades, with chromosomal *cpe+* strains in one clade and plasmid *cpe+* and *cpe-* in the other clade. Comparative genomic analysis carried out on core genes identified 85 genes specific to *cpe+* strains and 132 genes specific to *cpe-* strains. Pan- and core-genome analyses revealed the conservation of 6 % of the genome (core-genome) throughout the 146 strains, with a large and open pan-genome. This pan-genomic feature enables different strains to survive in diverse environmental niches.

Genome scanning for mobilome identified five different types of plasmids described as carrying toxins and antibiotic resistance genes. In relation to antibiotic resistance genes. Analysis of the resistome indicate the presence of *mprF* gene in 146 strains of the collection. Potential resistance to tetracycline (*tet(A)P* and *tet(B)P*) and to bacitracin (*bcr*) was identified indicating a risk of resistance among *C. perfringens* strains involved in FBOs. Analysis of the mobilome identified the presence of phages in 73 % and CRISPR-Cas systems in 64 % of isolates studied. This high presence of phages results from the diversification of the host and the adaptation of *C. perfringens* to different ecological niches.

In conclusion, WGS analysis allowed for a better toxicic characterization of *C. perfringens* by determining virulence gene profiles at the genomic level and by identifying mobile genetic elements of *C. perfringens* strains involved in FPO. ■



## Jin-Hee HWANG / National Institute of Food and Drug Safety Evaluation (NIFDS), Ministry of Food and Drug Safety, South Korea

Dr. Hwang is Deputy director of Division of Food Microbiology, Department of Food Safety Evaluation, National Institute of Food and Drug Safety Evaluation (NIFDS), Ministry of Food and Drug Safety (MFDS). She has been with the Korea Ministry of Food and Drug Safety (MFDS) since 1995, and she has recently been joined the Division of Food Microbiology since February 2018. She is also assigned as a Team Leader for Foodborne Diseases Investigation and Research TF Team. She received her Ph. D. in Microbiology from the Chung-Ang University, Republic of Korea in 2004. She is involved in research in investigation of foodborne diseases and whole genome sequencing analysis of foodborne pathogens.

### Foodborne outbreaks investigation and whole genome sequences analysis in Korea MFDS

Food safety management is important issue because of growing the consumer interests in foods according to the advanced society and change of diet. Also food poisoning incidents associated food safety constantly occurs when people ate contaminated food. For more than 20 years, pulsed-field gel electrophoresis (PFGE), a molecular fingerprinting technique, was the principal method for detecting and investigating foodborne disease outbreaks.

However, PFGE has limitation because of the detection method based on frequencies and locations of restriction enzyme sites on the bacterial genomic DNA. And, identification of reason on foodborne disease is difficult since new and genetic variation species appeared. Therefore, it is necessary to study whole genome sequence analysis of foodborne pathogenic using next-generation sequencing (NGS). In this study, we investigated whole-genome sequencing (WGS) analysis to identify the reason of foodborne pathogenic bacteria. Our data set consisted of 70 *Salmonella enterica* isolated obtained from clinical and food sources of reported foodborne outbreaks in the Republic of Korea during the period 2014-2016.

We compared pulsed-field gel electrophoresis (PFGE) band patterns with next-generation sequencing based single nucleotide polymorphism (SNP) patterns. Result showed that SNP patterns were clearly separated rather than PFGE patterns. In conclusion, this study demonstrates that next-generation sequencing based single nucleotide polymorphism (SNP) provide the resolution and accuracy needed for outbreak investigations of food-borne pathogens. ■



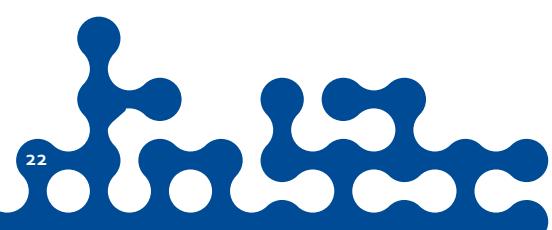
WEDNESDAY 27 MARCH

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# SESSION 3

## Quantitative risk assessment modelisation and source attribution in the genomic era

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## CHAIRS

**Mirko ROSSI** / European Food Safety Authority (EFSA), Italy

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*Doctor in Veterinary Medicine with a PhD in Epidemiology and control of Zoonoses from University of Bologna, he received the title of Docent in Zoonotic Bacteriology from the University of Helsinki in 2015. From 2013 to 2018 he was appointed as associate professor at the University of Helsinki researching on genomic epidemiology of Campylobacter and other food-borne pathogens. Currently he is scientific officer at the European Food Safety Authority, Unit Biological Hazards and Contaminants.*

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**Gilles SALVAT** / ANSES, France

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*Doctor of Veterinary Medicine (DVM) and PhD, with more than 30 years of experience in the management of scientific teams, General inspector of veterinary public health, scientist and expert on food microbiology and animal health and welfare, Gilles Salvat authored or co-authored more than 240 international publications and conference communications, including nearly 100 referenced in PubMed and/or Scopus (H-Index: 29). As a researcher, he developed his research in the field of foodborne zoonosis with a special reference on poultry and swine production and on *Salmonella*, *Listeria monocytogenes*, and *Campylobacter*. After directing a research unit on poultry and swine foodborne zoonosis (1997-2004), he held the position of Director of one of the largest research laboratories of Anses, located in Brittany (Western France), from 2004 to 2018. He was nominated as the Scientific Director for animal health and welfare for all of ANSES in 2011. Since February 2018, he is Managing Director General of Research & Reference Division.*

*The Managing Director General for the Research and Reference Division coordinates the various Agency entities with activities in the areas covered by Anses nine laboratories: animal health & welfare, food safety, and plant health. This new position is an opportunity to promote translational research among Anses research and risk assessment scientific teams and to improve the organization of ANSES' nine laboratories with the aim of improving the Agency's capacity for answering the public health and animal health and welfare challenges.*

# FOODBORNE PATHOGENS & WHOLE GENOME SEQUENCING

JOINT CONFERENCE

## KEYNOTE SPEAKER

### Lapo MUGHINI-GRAS

/ National Institute for Public Health and the Environment (RIVM),  
Centre for Infectious Disease Control, Bilthoven, the Netherlands

*Lapo Mughini-Gras is epidemiologist at the RIVM and associate professor at Utrecht University. He is graduated in veterinary medicine (Bologna University, 2008), PhD-qualified (Istituto Superiore di Sanità, 2010-2013), and specialised in animal production (Perugia University, 2013-2015). His research focuses on zoonosis epidemiology within the One Health framework. He develops models to understand the sources, transmission routes, and consequences of these infections to provide a quantitative, risk-based evidence base that binds science to decision making. He is engaged in several teaching activities and workgroups. He is bilingual Italian and Spanish, and fluent in English, Dutch and French. Since 2010, he coauthored over 80 peer-reviewed papers.*

## Towards ‘next-generation’ source attribution and microbial risk assessment of foodborne pathogens

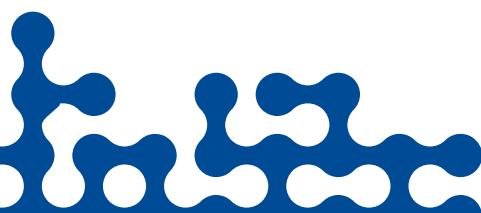
As the whole genome sequencing (WGS) revolution is rapidly gaining momentum, it is essential to understand the significance of this technology and its future applications in food safety, particularly its potentialities, hurdles, and recent advancements in source attribution and microbial risk assessment. Although WGS is increasingly being applied in infectious disease surveillance and outbreak investigation, there is still a strong need for harmonization of methods (e.g. sample preparation, sequence quality, case definition, etc.) and consensus on suitable nomenclature (e.g. SNPs vs. allele numbers).

The application of WGS in source attribution and microbial risk assessment is still in its infancy, but it has a great potential for change. The use of WGS in source attribution requires the development of new (or the drastic adaptation of) existing modelling approaches that can handle large amounts of high-throughput data, and therefore cope with the high discriminatory power associated with WGS. So far, efforts to quantify the relative contributions of different (animal, food, and environmental) sources to human illness have mostly relied on phenotyping methods, and on genotyping methods other than WGS (e.g. MLST). Defining the optimal discriminatory level for source attribution depends on the level of clonality and degree of host association of the pathogen in question.

There is often considerable uncertainty in source attribution for specific, relatively monomorphic, pathogen subtypes that are regularly isolated from different sources, and WGS can help identifying fine-scale genetic structures associated with host specificity. Ideally, source attribution methods should allow for some genetic diversity between isolates, but only to the degree that it can still be assumed that they originate from the same source. This requires a way to define host associations per locus, SNP, or groups of loci/SNPs.

A methodological shift towards the combined analysis of epidemiological and molecular-based source attribution data has also been envisaged to bridge the gap between attributing at the point of exposure (i.e. risk factors) and at reservoir level, thereby inferring the underlying transmission pathways.

Microbial risk assessment provides estimations of both the likelihood and the level of the microbial hazard in a specified food portion and considers microbial behaviour. While mostly phenotyping data are being used in exposure assessment, mechanistic cellular information, obtained using omics techniques, can enable the fine-tuning of exposure assessments. The link with phenotypic features is crucial, but the shortcomings regarding the reproducibility of genome-wide-association studies and the link to epidemiology need innovative statistical approaches. Overall, WGS data alone are of limited use without a sound public health or biological context.



The main challenge faced by microbial risk assessment when using WGS data is the issue of disaggregation, as the number of hazards to be considered increases exponentially when zooming in from phenotypes into genotypes.

The integration of WGS data in microbial risk assessment requires a paradigm shift with respect to the current risk assessment framework. This shift involves the translation of multidimensional genotyping data into reduced information on the phenotype and to ultimately generate a measure of risk that matches the requirements of food safety authorities. In order for WGS to be incorporated into risk assessment in a useful manner, priority setting of high-risk phenotypes is necessary. High levels of genome similarity do not imply similar behaviour in the food chain or similar levels of virulence since small genetic changes may result in large phenotypic differences. It is therefore of high importance to link genome sequences with phenotypic properties on persistence in the food chain and *in vitro* or *in vivo* virulence assessments.

This could help understanding why, for instance, the occurrence of certain types among human cases cannot be solely explained by differences in exposure (or host factors), but it may reflect differences in virulence factors conferring, e.g., persistence along the farm-to-fork continuum. In term of modelling approaches, without changing the usual model structure, caveats are expected. A 'next-generation' of source attribution and microbial risk assessment models exploiting all insights in the variability of a pathogen's genetic background and behaviour based upon omics techniques will increase our quantitative knowledge on the pathogenic strains. ■

## Laurent GUILLIER / ANSES, Food Safety Laboratory, Unit Salmonella and Listeria, France

*Laurent Guillier has an initial formation of food microbiologist engineer. He defended his PhD thesis in 2005 on predictive microbiology. To this date, I joined the INRA laboratory as research engineer where he conducted researches on modelling bacterial competition in biofilm and the activity of natural antimicrobials. From November 2006 to the end of 2009, he worked in the ANSES Risk assessment department where I contributed to more than 25 opinions of the French food safety agency on quantitative risk assessments, microbiology, hygiene, and sampling. Since that period, he continues to participate as a scientific expert for numerous opinions of ANSES, three for EFSA (meat transport) and one for FAO/WHO. Since 2010, he works in the Laboratory of Food Safety of Maisons-Alfort in the Department of microbiological contaminant. I was in charge of the scientific coordination of several national ANR projects. Since 2015, he has been involved in several European projects (BFR/DTUU/ANSES RAKIP project, EFSA CALL BIOCONTAM, H2020-COMPARE, EJPOH LISTADAPT). More specifically, he conducts project on modelling, bio-informatic analysis (microevolution, GWAS) and source attribution. Laurent Guillier published more than 65 scientific peer reviewed articles and gave more than 25 oral presentations in national or international conferences. I reviewed more than 60 articles (International Journal of Food Microbiology, Frontiers, Food Microbiology...).*

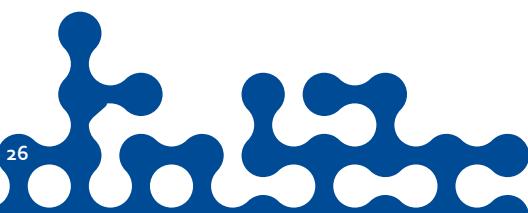
## The use of Whole Genome Sequencing in risk assessment: Application to the cold smoked salmon-related listeriosis risk model

Recent developments in genome sequencing open new opportunities for explaining the intraspecific variability of phenotypes (e.g. virulence, growth behavior). Successful association between WGS-data and specific phenotypes is thought to contribute to better predicting microbial behaviors.

Implementing this information in hazard identification, exposure assessment, and hazard characterization processes will refine quantitative microbial risk assessments (QMRA) models. The aim of this study was to explore the refinements in QMRA studies when considering pheno-genotype associations for the hazard properties, particularly related to the growth ability at low temperature (minimal growth temperature,  $T_{min}$ ) and the virulence. The used QMRA-model was previously developed for the assessment of the number of listeriosis cases associated to cold-smoked salmon in France.

The global prevalence in the existing model was replaced by the specific prevalence for each genotypic subgroup (clonal complex - CC) in Europe. In order to describe the variability of *Listeria monocytogenes*' growth characteristics more accurately, two different distributions of  $T_{min}$  were implemented. For risk characterization, three different groups of virulence were considered according to the CCs. Each group was associated with a specific dose-response model. The new QMRA model showed that CCs contributing the most in consumer exposure were not those that contributed the most to listeriosis cases. The most prevailing CCs led to few listeriosis cases, whereas uncommon high virulent strains were responsible for the majority of predicted cases.

Similarly, the less prevailing group of strains with high  $T_{min}$  was approximately two times less implicated when considering human listeriosis in comparison to food contamination. Considering genotypic data in QMRA opens the way for the establishment of risk based measures specific to distinct sub-groups of *L. monocytogenes*. ■



## Marianne CHEMALY / ANSES, Laboratory of Ploufragan-Plouzané-Niort, Unit of Hygiene and Quality of Poultry and Pork Products, France

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*Dr. Marianne Chemaly, PhD, HDR in Food microbiology, is the head of the HQPAP Unit in Anses and research director of projects related to the poultry production. Her research activities focus on the "control of zoonotic pathogens in poultry production using a multifactorial approach" and cover the global chain from farms to forks. The main topics deal with the prevalence and molecular epidemiology, Host-pathogen interactions and control measures. She has been involved in several EU projects and coordinated workpackages under different programs (FP6, FP7 and Emida Era-Net). She is actively involved in expertise through participation in different working groups as expert on Salmonella and Campylobacter at the national (Anses), EU (EFSA) and international (FAO/WHO) levels and is currently member of the EFSA panel on biological hazards "Biohaz".*

### Campylobacter: recent knowledge using genomics and metagenomics

*Campylobacter* is the most common cause of human intestinal infections of bacterial origin in the world. At the European level, more than 200 000 cases of campylobacteriosis are reported annually by the European Food Safety Authority (EFSA). Raw poultry products and cross-contaminations are the main risk factors for human infection. However, poultry does not cover all human cases since 50 to 80 % of cases would be attributed to the poultry reservoir and 20 to 30 % to poultry meat according to a quantitative risk analysis study carried out by EFSA. Identifying and estimating the involvement of other animal sources is crucial for adapting control methods and reducing human cases.

Several molecular genotyping approaches, in particular Multi-Locus Sequence Typing (MLST), have been used to describe *Campylobacter* populations improving the understanding of their epidemiology and genetic. MLST has been widely used to identify the origin of human campylobacteriosis based on allelic variations of 7 loci, and has identified poultry as a major source of infection in several countries. The increasing availability of bacterial genomes provides data on allelic variation of loci across the genome, improving the accuracy of source attribution. Using a pan-genome reference approach and a systematic gene-by-gene comparison of several hundred genomes of *C. jejuni*, 15 loci were chosen as markers for source attribution, as they allowed the segregation of isolates based on their host to identify the most likely origin of campylobacteriosis cases in France in 2009 and 2015. To this end, a collection of 1067 *C. jejuni* from chickens, ruminants, pets, environmental waters and clinical cases was characterized by MLST and a sub-selection of 370 isolates was sequenced by WGS.

Source attribution using the segregation markers of the host, showed that ruminants and chickens were equivalently involved in campylobacteriosis in 2009 while chicken was the primary source of infection in 2015. The use of the segregation markers of the host in *Campylobacter* source attribution study allowed a greater discrimination of chicken isolates. The cattle reservoir is found to be a significant route of transmission for *C. jejuni* to humans in France, and the important role of chicken in campylobacteriosis is confirmed.

On another level, control measures aimed at reducing the intestinal load of *Campylobacter* in poultry using feed additives, have not led to a systematic application on farms. In fact, the reductions observed during experimental trials were not reproducible at the field scale and the inter-individual variability did not allow to conclude to significant effects. In order to elucidate mechanisms that could influence and improve control measures, metagenomic approaches offer the possibility to study the involvement of gut microbiota in this system. The first elements of the analysis of chicken gut microbiota showed significant modifications of the caecal microbiota composition in animals that received feed additives compared to control animals, despite the absence of an effect on the level of contamination in *Campylobacter*. A better understanding of the role of the microbiota in host-pathogen interactions would make it possible to propose solutions to control the colonization of chicken by *Campylobacter*. ■



### Matthias FILTER / Federal Institute for Risk Assessment (BfR), Germany

*Matthias Filter (BfR) is senior research scientist in the unit "Food Technologies, Supply Chains and Food Defense" at the German Federal Institute for Risk Assessment (BfR). Matthias holds a diploma in Biochemistry and has been working as research scientist and project manager in public and private sector organizations for more than 20 years. He coordinated several software development and research activities in national and international research projects.*

### RAKIP: Resources for harmonized annotation and efficient exchange of risk assessment models

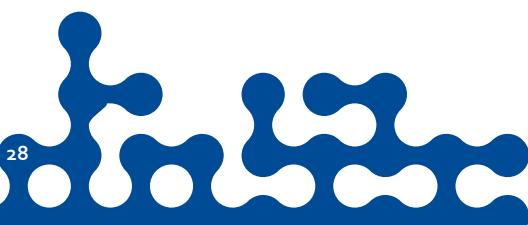
Virginie Desvignes/ANSES, France ; Laurent Guillier/ANSES, France ; Maarten Nauta/National Food Institute, DTU, Denmark

Risk assessment in the food safety domain is an interdisciplinary effort relying on integration of current scientific knowledge available in a variety of information sources, e.g. scientific publications, experimental data, databases, mathematical models and software tools. However, the extraction and exchange of information from and between these resources is frequently difficult and time consuming. This is true, despite the fact that digital technologies already facilitated large progress in the way how information can be made available and shared.

In order to establish new community resources facilitating the efficient knowledge integration and exchange into and between IT-based applications, the three agencies ANSES, BfR and DTU Food initiated the "Risk Assessment Modelling and Knowledge Integration Platform" (RAKIP) project. Through the joint development of harmonized data formats and rules for knowledge annotation, this project laid the foundation for the first technical implementation of a RAKIP Portal that is available at <https://foodrisklabs.bfr.bund.de/rakip-web-portal/>. This portal encompasses a proof-of-concept community model repository and provides supporting resources facilitating efficient knowledge exchange in the future. The RAKIP Model Repository (<https://foodrisklabs.bfr.bund.de/rakip-model-repository-web-services>) allows users to access, search, filter, execute and download risk assessment models or parts thereof in the new file format "Food Safety Knowledge Markup Language" (FSK-ML).

These FSK-ML formatted model files can then be imported and executed by other software tools supporting this information exchange format, e.g. the open source desktop application KNIME / FSK-Lab. In this way RAKIP created the basis for more efficient knowledge sharing within the Quantitative Microbial Risk Assessment (QMRA) and predictive microbial modelling community as existing models and data can now easily be shared and re-used. For example, specific process models, dose-response models, or complete QMRA models can be adapted to specific needs or used as building blocks in new risk assessments.

Given these very promising results, it is reasonable to explore whether the RAKIP concept and FSK-ML can be adapted (and linked with existing resources) to serve the needs of novel risk assessment approaches, as e.g. those applying NGS / WGS data. ■



WEDNESDAY 27 MARCH

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# SESSION 4

## Pipelines and workflows for WGS data analysis

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# FOODBORNE PATHOGENS & WHOLE GENOME SEQUENCING

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## CHAIRS

**Soohwan SUH** / National Institute of Food and Drug Safety Evaluation (NIFDS),  
Ministry of Food and Drug Safety, South Korea

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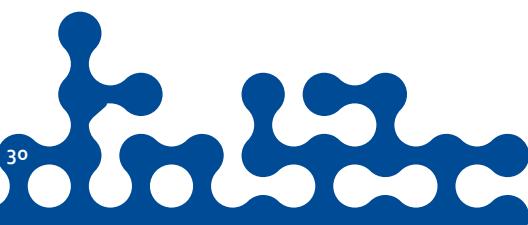
*Dr. Suh is a research microbiologist. He has been with the Korea Ministry of Food and Drug Safety (MFDS) since 2013, has been with Division of Food Microbiology, Department of Food Safety Evaluation, National Institute of Food and Drug Safety Evaluation (NIFDS), Ministry of Food and Drug Safety (MFDS). He completed his Ph. D. training at the North Carolina State University (NCSU), USA in 2012. His postdoctoral training took place with the USDA "NOROCORE food virology collaborative for outreach, research and education" during 2012-2013. His research interests include: developing probabilistic models to characterize exposure and risk associated with microbial hazards in food; Monitoring and characterization of antimicrobial resistance of bacteria from livestock products; Study of the survival mechanisms and genetic characteristics of anti-acidic pathogenic Escherichia coli; and development of novel bio-ligands such as DNA/RNA aptamers and peptide nucleic acid for the detection and inactivation of pathogenic organisms.*

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**Eric BROWN** / Center for Food Safety and Applied Nutrition, Food and Drug Administration, USA

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*Dr. Eric W. Brown has been with the Food and Drug Administration's Center for Food Safety and Applied Nutrition (CFSAN) since 1999 and currently serves as Director of the Division of Microbiology in the Office of Regulatory Science. Here, he oversees a group of 60 food safety microbiology researchers and support scientists engaged in a multi-parameter research program to develop and apply microbiological and molecular genetic strategies for detecting, identifying, and differentiating bacterial foodborne pathogens such as *Salmonella*, *Listeria*, and shiga-toxin producing *E. coli*. Recently, his laboratory has been instrumental in adapting next-generation sequencing technologies to augment foodborne outbreak investigations and to ensure preventive control and compliance standards at the FDA including the establishment of the GenomeTrakr whole-genome sequencing network for food safety. Dr. Brown received his M.Sc. in Microbiology from the National Cancer Institute/Hood College joint program in the biomedical sciences in 1993 and his Ph.D. in Microbial Genetics from The Department of Biological Sciences at The George Washington University in 1997. He has conducted research in microbial evolution and genetics as a research fellow at the National Institutes of Health, the U.S. Department of Agriculture, and as an Assistant Professor of Microbiology at Loyola University of Chicago. He has been a member of the American Society for Microbiology since 1994 and was inducted as a Fellow of the American Academy of Microbiology in 2015. He has co-authored more than 200 refereed publications and book chapters on the molecular differentiation, evolutionary genetics, and ecological persistence of bacterial pathogens.*



## KEYNOTE SPEAKER

**Mirko ROSSI** / European Food Safety Authority (EFSA), Italy

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*Doctor in Veterinary Medicine with a PhD in Epidemiology and control of Zoonoses from University of Bologna, he received the title of Docent in Zoonotic Bacteriology from the University of Helsinki in 2015. From 2013 to 2018 he was appointed as associate professor at the University of Helsinki researching on genomic epidemiology of Campylobacter and other food-borne pathogens. Currently he is scientific officer at the European Food Safety Authority, Unit Biological Hazards and Contaminants.*

## Integration of genomics in outbreak detection and investigation of foodborne pathogens: state-of-the-art and challenges

Implementation of whole genome sequencing (WGS) as molecular typing technique has shown clearly its strength to enhance laboratory-based surveillance of communicable diseases at the local, national and international levels. Genome-based typing of foodborne pathogens is replacing traditional analyses in several countries, revolutionizing outbreak detection and investigation, and becoming a relevant tool for control-oriented surveillance.

As 'one-stop-shop' for rapid pathogen characterization, a fully and functional implementation of WGS in public health and food safety microbiology is allowing a significant simplification of the analytical framework with the consequent reduction of human intervention and overall costs. Moreover, by being compatible with machine-to-machine communication and other eHealth solutions, WGS has the great potential to facilitate an effective interoperability across disciplines. Genomics introduces also new opportunities for using isolate information (metadata).

With WGS it is now possible to combine efficiently real-time high-resolution typing reflecting clonal relationship and evolution among the isolates, together with epidemiological and clinical data in a way that was not possible before. However, the road towards a wide consensual use of WGS in foodborne pathogen surveillance is not free from challenges. Despite the current distribution of next-generation technology being capillary, allowing nowadays the sharing of hundreds of thousands of sequences, there are noticeable variations between and within countries in the ability to translate genomic data into useful information for public health decision-making and food risk managers.

I will summarize the current state of the art of the use of WGS in foodborne pathogen surveillance and outbreak detection and investigation, introducing the basics of the methodologies currently in use for WGS-based typing and reviewing the existing bioinformatic solutions. Using several examples food-borne outbreak investigation at the EU level, I will highlight the main challenges related to the application of WGS including standardization and harmonization of the process, validation of epidemiological concordance and the development of a plain language. Finally, I will present the activities currently ongoing at the European Food Safety Authority regarding the implementation of WGS in the context of outbreak investigations. ■



### Simon TAUSCH / Federal Institute for Risk Assessment (BfR), Germany

*Simon Tausch is staff bioinformatician at the BfR. In his PhD Thesis, he developed several bioinformatic tools related to metagenomics and NGS based pathogen diagnostics. In his current position, he develops data-management solutions and efficient pipelines for real-time analyses of food-related bacteria and conducts research on metagenomics.*

### PathoLive - Pathogen detection while sequencing

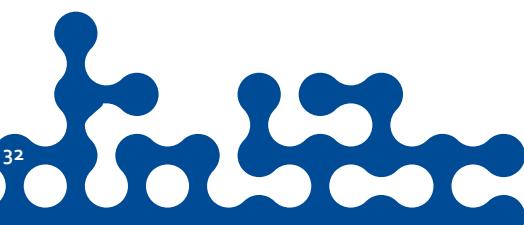
Over the past years, NGS has been applied in time critical applications such as outbreak analyses and pathogen diagnostics with promising results. Yet, long turnaround times have to be accepted to generate sufficient data, as the analysis can only be performed sequentially after the sequencing has finished.

The interpretation of results can be further complicated by various types of contaminations, clinically irrelevant sequences such as commensal organisms, and the sheer amount and complexity of the data. We designed and implemented PathoLive, a real-time pipeline which allows the detection of pathogens from samples up to several days before the sequencing procedure is even finished and currently available tools may start to run. We adapted the core algorithm of HiLive, a real-time read mapper, and enhanced its accuracy for our use case.

Furthermore, common contaminations, low-entropy areas, and sequences of widespread, non-pathogenic organisms are automatically marked beforehand using NGS datasets from healthy humans as a baseline. The results are visualized in an interactive taxonomic tree that provides an intuitive overview and detailed measures regarding the relevance of each identified potential pathogen. We applied the pipeline on a human plasma sample that was spiked in vitro with vaccinia virus, yellow fever virus, mumps virus, Rift Valley fever virus, adenovirus, and mammalian orthoreovirus.

The sample was then sequenced on an Illumina HiSeq. All spiked agents were detected after the completion of only 12% of the sequencing procedure and were ranked more accurately throughout the run than by any of the tested tools on the complete data. We also found a large number of other sequences and these were correctly marked as clinically irrelevant in the resulting visualization. This tagging allows the user to obtain the correct assessment of the situation at first glance. ■

PathoLive is available at [https://gitlab.com/rki\\_bioinformatics/PathoLive](https://gitlab.com/rki_bioinformatics/PathoLive)



## Nicolas RADOMSKI / ANSES, Food Safety Laboratory, Genome Analysis Modelling Risk (GAMeR), France

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*Nicolas Radomski has a background spanning conventional microbiology to genomic techniques, related to food microbiology, environmental risk assessment, control of zoonotic infections and public health. After completing a PhD from "École des Ponts ParisTech" (France) and "Virginia Polytechnic Institute" (USA), as well as a postdoctoral position from "Research Institute of McGill University Health Center" (Canada), he is now in charge of research projects related to bacterial genomics in Anses.*

### Adaptation to animal sources of *Salmonella enterica* subsp. *enterica* deciphered by Genome Wide Association Study and Gene Ontology Enrichment Analysis at the pangenomic scale

*Salmonella enterica* subsp. *enterica* is a public health issue related to food safety, and its adaptation to animal sources remains poorly described at the pangenome scale. Genome Wide Association Study (GWAS) from human genetics has recently been successfully adapted to bacteria to decipher the genetic determinants of host speciation, antibiotic resistance and virulence. Within the framework of the PhD degree of Meryl Vila Nova, the combination of GWAS with Gene Ontology Enrichment Analysis (GOEA) will allow elucidating the genetic and metabolic signatures related to the adaptation to animal sources.

As a first step, serovars presenting potential mono- and multi-animal sources were selected from a curated and synthetized version of the database Enterobase, and the corresponding sequencing reads were downloaded from the European Nucleotide Archive (ENA) (i). In a second phase, the accessory genes and coregenome variants were detected by pangenome extraction and variant calling analysis, respectively (ii). Thirdly, the accessory genes, as well as single nucleotide polymorphisms (SNPs) and small insertions/deletions (InDels) from the coregenome, were associated to animal sources based on a GWAS correcting the population structure with a linear mixed model (iii). Lastly, a GOEA integrating the most recent parent-child approach was applied to emphasize metabolic pathways mainly impacted by the pangenomic mutations associated to animal sources (iv).

Based on a curated and synthesized version of Enterobase, we established a dataset of 440 paired-end sequencing reads from ENA, representing 15 *Salmonella* serovars with associated spectra of animal sources, from generalist (i.e. multi-animal sources) to highly preferential (i.e. mono-animal sources) (i). Overall, 19 131 accessory genes and 159 221 coregenome variants were detected. The coregenome was constituted of 2 705 coregenes and 3 030 homologous recombination events in inter- and intra-genic regions (ii). Excluding variants from homologous recombination events, these accessory genes and coregenome variants were tested by GWAS to retain 314, 708, 587 and 361 mutations potentially associated to avian, bovine, swine and fish sources (Wald tests;  $p < 1 \times 10^{-2}$ ) (iii). These associated mutations were assigned to the corresponding metabolic pathways by GOEA (Hypergeometric tests;  $p < 5 \times 10^{-2}$ ) (iv).

We developed an integrated approach to screen pangenomic signatures of *Salmonella enterica* subsp. *enterica* associated to animal sources. The combination of a statically supported dataset of genomes with GWAS and GOEA, allowed deciphering the mutations and metabolic pathways related to the adaptation to animal sources. ■



## Pimlapas LEEKITCHAROENPHON / National Food Institute, DTU, Denmark

Researcher, PhD. my research expertise includes whole genome sequencing (WGS) and epidemiology, evolution in bacterial genomes and population structure of foodborne pathogens. I have extensive experience in applying WGS in food safety and public health protection with the main focus on antimicrobial resistance. Some of my current projects include WGS analysis within the EU Reference Laboratory for AMR, the EU Horizon 2020 COMPARE project on source attribution using machine learning, and the Novo Nordisk Foundation project on AMR. In addition, I facilitated and conducted international bioinformatics training courses in WGS data analysis including online course on WGS analysis in COURSERA.

### First steps towards incorporation of whole-genome sequencing data in exposure assessment: Machine Learning and Network-Diffusion approaches

P. Leekitcharoenphon / National Food Institute, DTU, Denmark ; AR. Rebelo / National Food Institute, DTU, Denmark ; LT. Hansen / National Food Institute, DTU, Denmark ; RS. Hendriksen / National Food Institute, DTU, Denmark ; T. Hald / National Food Institute, DTU, Denmark ; M. Bersanelli / Department of Physics and Astronomy, University of Bologna ; E. Mosca / Institute of Biomedical Technologies, National Research Council, Italy

Exposure assessment of microbiological hazards in food is a major part of the four components of the microbial risk assessment (MRA) process. This step involves the assessment of the qualitative and/or quantitative likely intake of microbial risk agents via food or other relevant sources. The physiological properties of the organism, extrinsic factors such as the environmental conditions (temperature, humidity..) as well as intrinsic properties (physical and chemical properties e.g. pH, water activity, ..) affect microbial survival, growth and/or death. Despite gains in accurate subtyping of microbial hazards using high-throughput DNA sequencing technologies, the incorporation of this data in exposure assessment during MRA remains an unexplored area in the public health domain and has only recently received scientific attention and proposals for future steps with no reports of application in a practical framework. In this presentation, approaches will be presented incorporating machine learning (ML) and network-diffusion based analysis of *Listeria monocytogenes* genomic profiles for exposure assessment considering response to the four stress conditions: desiccation, salt concentration, pH and low temperature storage.

Histograms of maximum growth rates of *Listeria* for the four stress conditions indicated multi-modality which cannot easily be described by standard distributions. Inference for these mixed populations of *Listeria* stress tolerance was made using finite mixture models to reveal underlying categories, proportion of the population of isolates in each particular tolerance category and to classify each of the isolates into a category of the mixture. This yielded categories such as highly susceptible, susceptible, tolerant and highly tolerant for acid stress. With such categories in mind, further interest was in making predictions about the phenotype category using the genotype. Total of 7348 accessory genes in amino acid sequences were used as model input to predict and differentiate each of the stress categories using supervised ML models. A matrix of percent similarity between accessory genes and the *Listeria* genomes was generated and subsequently used as input for ML. ML algorithms random forest, support vector machine (radial kernel, linear kernel) gradient boosting, logit boost and neural network were evaluated for their prediction accuracy with 10-fold cross validation. Random forest was chosen as appropriate model for the cold (Accuracy: 96%; CI: 90-99%), salt (Accuracy: 86%; CI: 79-92%) and desiccation stress (Accuracy: 91%; CI: 85-96%) responses although it performed as good as other choices for some stress responses. Support vector machine (radial kernel) (Accuracy: 88%; CI: 81-94%) emerged as best model in prediction of acid stress response. Top genes contributing to the predictions were selected.

The genes characterizing the genomic shift from tolerant to sensitive and conversely from sensitive to tolerant from a network perspective may be connected or disconnected. Disconnection means that an area of the protein-protein interaction has a specific role for the characterization of the genomic profile toward specific class (susceptible or tolerant), while connection means that a certain area of the network is suitable for characterizing the overall shift. Network diffusion based analysis of *Listeria* genomic profiles was conducted after mapping of genes to string identifiers. Out of 400 genes, 33 genes (17%) appeared in all 4 stress types, 30 genes (15%) in 3 out of 4 stress types, 49 genes (26%) in 2, while 80 genes were stress-phenotype specific. Among these the major specific contribution was given by desiccation and salt stress phenotypes. Next steps towards incorporation into microbial risk assessment, validation and interpretation of completed modelling outputs will be presented. ■

WEDNESDAY 27 MARCH

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# SESSION 5

## Antimicrobial resistance

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# FOODBORNE PATHOGENS & WHOLE GENOME SEQUENCING

JOINT CONFERENCE

## CHAIRS

Jean-Yves MADEC / ANSES, France

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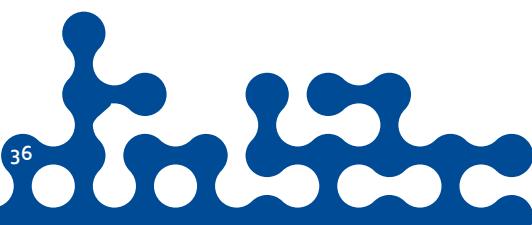
*Jean-Yves Madec is DVM, PhD, molecular microbiologist, Scientific Director on AMR at Anses and Head of the Research Unit "AMR and Virulence" (Anses Lyon). JYM coordinates the Resapath network monitoring AMR in clinical animals in France. His research interests focus on molecular genetics and epidemiology of AMR, including its cross-sectorial spread in a One Health perspective. JYM is a member of several expert groups on AMR and an active participant/leader in European/transnational scientific projects.*

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Valeria BORTOLAIA / National Food Institute, DTU, Denmark

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*Dr. Valeria Bortolaia is a Senior Researcher at the Technical University of Denmark where she works in the field of antibiotic resistance at the human-animal health interface. Her scientific goal is to limit spread of antibiotic resistance thus preserving our ability to treat infectious diseases. Dr. Bortolaia's research targets the dynamics of resistance transfer among bacteria from animals, food and humans and the development of genomic-based tools for rapid detection of antibiotic resistance.*



## KEYNOTE SPEAKER

### Rene Sjøgren HENDRIKSEN / National Food Institute, DTU, Denmark

*Dr. Rene S. Hendriksen is professor at the Technical University of Denmark, National Food Institute and act as director and deputy for the reference centres; World Health Organization Collaborating Centre (WHO CC) for Foodborne Pathogens and Genomics and the European Union Reference Laboratory in Antimicrobial Resistance (EURL AR), respectively. He provide advisory service to European Commission, European Food Safety Authority, WHO Global Foodborne Infection network, WHO Global Antimicrobial Resistance Surveillance System, WHO Advisory Group on Integrated Surveillance of Antimicrobial Resistance, Food and Agriculture Organization of the United Nations in the area of antimicrobial resistance and whole genome sequencing. His main focus is research in global epidemiology, surveillance, antimicrobial resistance, and population structure of mainly food and waterborne pathogens. He has been working with the most important food and waterborne pathogens including a long range of *Salmonella* serovars including *S. Typhi*, *Campylobacter* spp., *E. coli*, *Listeria monocytogenes*, *Shigella* spp., *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Vibrio cholerae*, and *Enterococcus* spp.. Since 2010, the Research Group has embraced the new era of whole genome sequencing and lately also metagenomics with WHO and FAO supported initiative, "the Global Sewage Surveillance project" using a metagenomics approach to establish a global surveillance of human infectious diseases including antimicrobial resistance from sewage collected in major cities around the world to detect, control, prevent and predict human infectious diseases. Rene S. Hendriksen is author of 102 peer-reviewed published and accepted articles in international refereed journals conducted in collaboration with >500 scientists in >100 countries*

## The presence and future in antimicrobial resistance surveillance

Humans and animals are exposed more than ever to the emerging threat of antimicrobial resistant (AMR) bacterial pathogens due to increased globalization, urbanization, poverty, poor sanitation, climate change, population growth and agricultural intensification.

For decades, phenotypic antimicrobial susceptibility testing (AST) have been applied and are widely accepted globally as the golden standard for AMR surveillance. Thus, WHO, FAO, NARMS, and EUSR amongst others have all been trying to establish various national, regional or global AMR surveillance programs. Many of these initiatives, however, have had little global effect because many laboratories around the world; mainly in the developing part still provide doubtful phenotypic AST data despite many efforts to build capacity. In 1995, the first two complete bacterial genome sequences were published and whole genome sequencing (WGS) has since changed the landscape of microbiology. The recent advancements in rapid and affordable DNA sequencing technologies, along with online bioinformatics tools for real time genomic identification has revolutionized conventional microbiology and diagnostics making WGS a viable routine solution for epidemiological tracking and surveillance of bacterial pathogens and associated AMR determinants.

A handful of bioinformatics tools to predict AMR determinants have been developed which include among others ARG-ANNOT, CARD, SRST2, Genefinder, ARIBA, KmerResistance, and ResFinder. Several studies have already provided evidence of a high concordance, >96% between phenotypic AST data and predicted AMR as well as high levels in sensitivity, >87% and specificity, >98% depending of the species analysed. All of the tools have, however, also their individual strengths and limitations in usability and detection of AMR determinants highlighted in several benchmarking exercises and scientific articles.

Currently two networks exist, the Resistome Tracker from the US FDA and the EU COMPARE Pathogens portal from European Bioinformatics Institute, both to utilize WGS data as the basis for rapid and comprehensive sharing of bacterial pathogen identifications and associated AMR determinants to provide a global surveillance.

In the future, the surveillance system for AMR is likely be expanded by the introduction of metagenomics to potentially establish a global surveillance to detect and analyse the presence of all pathogens and associated AMR determinants among the healthy population of humans using urban sewage or / and the animals at farm level using faeces. The advancement in WGS and the application of online tools for real-time detection of AMR determinants are believed to be essential in the future efforts to control and develop prevention strategies to combat the increasing threat of AMR. ■



## Maria BOROWIAK / Federal Institute for Risk Assessment (BfR), Germany

Maria Borowiak is a molecular biologist at the German Federal Institute for Risk Assessment. She currently works in the department biological safety as a specialist for the characterisation of food-borne pathogens using WGS. Here, she is responsible for development and validation of WGS protocols. In her ongoing PhD project, she is interested in the characterisation of colistin- and carbapenem-resistant *Salmonella* isolates using WGS.

### Plasmid-mediated colistin resistance in German *Salmonella enterica* strains isolated from livestock, food and the environment

The polymyxin antibiotic colistin is considered as last-line treatment option for severe human infections. Resistance against colistin was for a long time linked to point mutations in chromosomally encoded genes. However, in 2015 the first mobile plasmid-located colistin resistance gene (*mcr-1*) was described. Since then, seven additional *mcr genes* (*mcr-2* to *8*) were identified.

For a detailed prevalence study on all known *mcr genes*, an extensive PCR screening was carried out including 446 colistin-resistant (MIC >2 mg/L) *Salmonella enterica* isolates from the strain collection of the National Reference Laboratory for the Analysis and Testing of Zoonoses (*Salmonella*) in Germany. The isolates, which were collected between 2011 and 2018, originate from food producing animals and food products. Excluded from the study were *Salmonella* isolates of serogroup D, which often show an intrinsic resistance to colistin. For detection of *mcr-1* to *mcr-5*, the multiplex PCR assay developed by Rebelo et al. was used. Screening for *mcr-6* to *mcr-8* genes was carried using PCR protocols developed in-house.

PCR results showed, that *mcr-1*, *mcr-4* or *mcr-5* could be detected in 287 colistin-resistant isolates. Remaining *mcr*-variants were not detected. The *mcr-1* gene is the most common colistin resistance gene found in *Salmonella enterica*, followed by *mcr-4* and *mcr-5*. While the number of *mcr-1*-positive isolates remains relatively stable since 2011, only few *mcr-4*-positive and no *mcr-5*-positive isolates were detected in the last two years. Most of the *mcr*-positive isolates could be assigned to specific *Salmonella* serovars including Heidelberg, Infantis, Newport, Paratyphi B dTa+, Saintpaul, Schwarzengrund, Typhimurium, Ohio and Rissen. Surprisingly the cause for colistin resistance remains to be explored in 36 % of the analysed isolates.

In this study the *mcr-5* gene was of special interest, since this gene was originally detected in colistin-resistant *Salmonella* Paratyphi B dTA+ isolates from the NRL *Salmonella* strain collection in Germany in 2017. In-depth WGS sequence analysis of these strains revealed, that the *mcr-5* gene was associated with a Tn3-family transposon, later termed as Tn6452. An *in situ* analysis of sequence data provided in the NCBI database revealed that this *mcr-5*- harbouring transposon or parts thereof are present in various Proteobacteria from different places around the world. A global spread of *mcr-5* is thus highly likely and further supported by a number of recent publications. The extensive PCR screening in the NRL *Salmonella* strain collection revealed that *mcr-5* is not only present in *Salmonella* Paratyphi B dTA+, but also in *Salmonella* Typhimurium. In this serovar, three novel *mcr-5* harbouring plasmids were identified and described using WGS. ■

**Soohwan SUH** / National Institute of Food and Drug Safety Evaluation (NIFDS),  
Ministry of Food and Drug Safety, South Korea

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Dr. Suh is a research microbiologist. He has been with the Korea Ministry of Food and Drug Safety (MFDS) since 2013, has been with Division of Food Microbiology, Department of Food Safety Evaluation, National Institute of Food and Drug Safety Evaluation (NIFDS), Ministry of Food and Drug Safety (MFDS). He completed his Ph. D. training at the North Carolina State University (NCSU), USA in 2012. His postdoctoral training took place with the USDA "NOROCORE food virology collaborative for outreach, research and education" during 2012-2013. His research interests include: developing probabilistic models to characterize exposure and risk associated with microbial hazards in food; Monitoring and characterization of antimicrobial resistance of bacteria from livestock products; Study of the survival mechanisms and genetic characteristics of anti-acidic pathogenic *Escherichia coli*; and development of novel bio-ligands such as DNA/RNA aptamers and peptide nucleic acid for the detection and inactivation of pathogenic organisms.

## One Health Approach on AMR surveillance in Korea

Antimicrobial resistance (AMR) poses the threat to human health worldwide. It is recognized that food plays an important role in the development and spread of AMR. Korean government has tried to slow down AMR by 'National Antimicrobial-Resistance Management Program (NARMP)' since 2003. Ministry of Food and Drug Safety (MFDS) continued to monitor AMR in retail meats as a part of NARMP.

The prevalence of bla<sub>CTX-M</sub> gene harboring *Salmonella enterica* serotype Virchow from human and meat samples has increased in Korea since the first isolation in 2011. This talk will highlight the characteristics of bla<sub>CTX-M</sub> gene harboring *Salmonella enterica* from meat samples and genetic relation between isolates from human samples and meat samples. ■



## Isabelle KEMPF / ANSES, Ploufragan-Plouzané-Niort Laboratory, France

Isabelle Kempf, DVM, PhD in microbiology, is head of the Mycoplasmology, Bacteriology and Antimicrobial Resistance Unit in the Ploufragan-Plouzané-Niort Laboratory of Anses. Since 2000, she works on antimicrobial resistance of zoonotic, pathogenic or commensal bacteria of poultry and pigs and their environment. She belongs to the French NRL for antimicrobial resistance in animals. She authored more than one hundred peer-reviewed articles.

### WGS and antimicrobial resistance

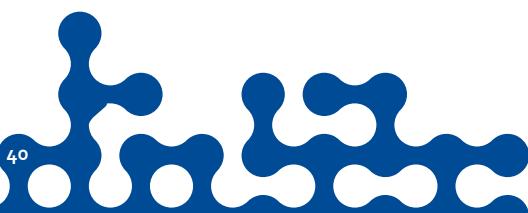
Antimicrobial resistance is a major health issue. Extended-spectrum cephalosporins (ESC) are critically important antimicrobials for human medicine. Resistance to ESC is encountered in commensal or pathogenic *Enterobacteriales* isolated from animal, human and environment origins. ESC resistance in animals in Europe is most commonly due to conjugative plasmids carrying extended-spectrum beta-lactamase (ESBL) and AmpC-encoding genes. In this presentation, we show how whole genome sequencing (WGS) can help us to investigate the diversity of the ESC-resistance genes and their genetic supports in bacteria of animal origins. WGS also enables the detection of the genes coding for resistance to other antibiotics, which may result in co-selection of these multidrug resistant bacteria. Virulence-associated genes present on ESC-resistance plasmids or genomes can also be searched.

Thus for example, we could observe that ESCR in French poultry and pigs is mainly carried by highly similar *bla<sub>CTX-M-1</sub>* *Incl1*/ST3 plasmids (Lucas et al. 2018, Touzain et al. 2018). The poultry *bla<sub>CTX-M-1</sub>* *Incl1*/ST3 plasmids often bear *sul2* and *tet(A)* and less often *bla<sub>TEM-1B</sub>*, *dfrA1*, *dfrA17* or *aadA5*, whereas the pig *bla<sub>CTX-M-1</sub>* *Incl1*/ST3 plasmids often bear *sul2*, *dfrA17* and *aadA5*, and occasionally *tet(A)*, *mph(A)*, *erm*, *floR* or *strA*, *strB*. Importantly resistance genes for quinolones or polymyxins were not detected and only few virulence genes (including colicins, heat-stable enterotoxins, adhesins or temperature-sensitive hemagglutinins) were present on these plasmids.

WGS also offers us the possibility to investigate the persistence and the diffusion of resistance genes, plasmids or bacteria at different scales, in field studies (Baron et al. 2018) or in experimentally inoculated animals. Thus in pigs, the rare in vivo loss of a multidrug resistance *Incl1*/ST12 plasmid, pESCR containing *bla<sub>CTX-M-1</sub>*, *bla<sub>CMY-2</sub>*, *sul2*, *dfrA17*, and *aadA5* genes, could also be demonstrated using WGS (Mourand et al. 2016). ■

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THURSDAY 28 MARCH

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# SESSION 6

## Metagenomics for food safety

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# FOODBORNE PATHOGENS & WHOLE GENOME SEQUENCING

JOINT CONFERENCE

## CHAIRS

**Carlus DENEKE** / Federal Institute for Risk Assessment (BfR), Germany

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*Carlus Deneke obtained his PhD at the Max-Planck-Institute for Colloids and Interfaces on modelling gene regulation and mRNA decay. At the Robert-Koch Institute he developed machine-learning based methods for the prediction of pathogenic bacteria and novel virulence factors. Since 2017 he works for the BfR in the Study Centre for Genome Sequencing and Analysis . His main tasks involve bioinformatic pipeline development, NGS data management and data analysis.*

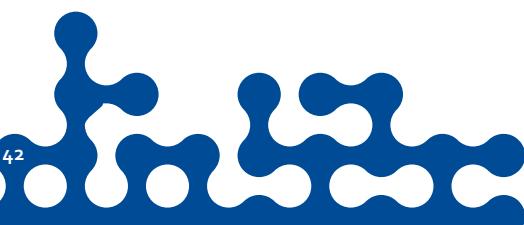
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**Michel-Yves MISTOU** / ANSES, France

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*Michel-Yves Mistou, research director at INRA (French national institute for agronomical research) joined ANSES in 2014 to take the head of the foodborne pathogens (FBP) department of the Laboratory for Food Safety (Maisons-Alfort). He has worked as a researcher in different institutions (CNRS, Max Planck Institut, INRA, Institut Pasteur) and has been project manager for multiple research projects involving national and international institutions.*

*He has defined the strategy and is managing the implementation of WGS technologies for research, reference and surveillance activities on foodborne pathogens in the FBP department and is responsible of the GAMeR team in charge of bioinformatics development in the laboratory for food safety. He is an expert at Anses, EFSA, ISO in the whole genome sequencing field and is the author of over 45 peer-reviewed published articles in the fields of enzymology, biochemistry, bacterial physiology, genetics and genomics.*



## KEYNOTE SPEAKER

Marion KOOPMANS /Erasmus MC, The Netherlands

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*Professor Marion Koopmans, DVM PhD focuses on global population level impact of rapidly spreading zoonotic virus infections, with special emphasis on foodborne transmission. Her research focuses on unravelling the modes of transmission of viruses among animals and between animals and humans, and the use of pathogen genomic information to unravel these pathways and to signal changes in transmission or disease impact. She is scientific coordinator of COMPARE, a large H2020 funded project (20 MEuro), exploring the potential uses of next generation sequencing techniques for outbreak detection and tracking ([www.compare-europe.eu](http://www.compare-europe.eu)), and co-PI in the FP7 funded PREPARE project ([www.prepare-europe.eu](http://www.prepare-europe.eu)) aimed at building a pan-European operational network for rapid and large-scale European clinical research in response to infectious disease outbreaks with epidemic potential. She is director of the WHO collaborating centre for emerging infectious diseases at Erasmus, and Scientific Director "Emerging infectious diseases" of the Netherlands Centre for One Health ([www.ncoh.nl](http://www.ncoh.nl)). She has received the Infectious disease award of the Dutch Association for Infectious Diseases and is the recipient of the Stevin Premium 2018. She has co-authored >500 papers that have been cited > 20.000 times.*

## Challenges and opportunities in foodborne virus genomics

Genome sequencing has been used extensively in research in the past 25 years to understand how viruses spread, to assess the contribution of food in their transmission, and to characterise sources of outbreaks. With the rapid development of lower cost sequencing platforms, the implementation of these technologies for real-time surveillance has been stated as a priority by national and supranational public health organisations.

Challenges to address are the lack of standardisation in the field, the fast evolution of technologies, and the need for increasingly complex bioinformatic analyses, with its own issues regarding standardisation. There is an increasing push for real-time open sharing of newly generated pathogen genome sequencing data on the one hand, but the need for some level of protection to safeguard against false positives which could erroneously point at a food source and recalls.

Despite these hurdles, it is clear that the technological revolution brings interesting opportunities to the field, that –when properly implemented- will help to improve our understanding of foodborne pathogen transmission. This presentation will review lessons learned on the use of pathogen genomics in the norovirus field, and discuss recent developments based on the COMPARE project. ■



### Josephine GRUETZKE / Federal Institute for Risk Assessment (BfR), Germany

*Josephine Gruetzke is a postdoctoral fellow at the BfR where she participates in the national funded Ess-B.A.R-project. Her project focusses on food safety and resilience of food supply chains in biohazard situations. Therefore, she develops and evaluates diagnostic procedures for the early detection of infectious zoonotic pathogens in the food chain using metagenomics. Beyond, she is primarily involved in the development of pipelines for the data analysis of metagenomic food samples.*

### Application of metagenomics for the detection of foodborne pathogens

Metagenomics is a valuable tool for microbial food safety because (i) it is universally applicable for the detection of all pathogens, (ii) it is fast since no isolation of microorganisms is required and (iii) it has a high resolving power on nucleic acid level available for pathogen characterization and source attribution. For undirected shotgun metagenomics total nucleic acids (NAs) are isolated from environmental samples such as foodstuff.

As a result along with microbial NAs high amounts of matrix NAs are extracted that in turn might outcompete microbial NAs during NGS and lead to a decreased sensitivity for the detection of low abundant microorganisms. Some pathogens like *Brucella* spp., a gram-negative biowarfare bacterium that can be transmitted via ingestion of contaminated dairy or meat products, feature a low infectious dose for humans estimated to 10 to 100 organisms and thus requires high-sensitive methods for its detection.

In our study we evaluated the ability of shotgun metagenomics for the detection and pathotyping of pathogenic bacteria as *Francisella tularensis* and *Brucella* spp. at different sequencing depths and bacterial count numbers by analyzing naturally and artificially contaminated foodstuff. ■

## Woojung LEE / National Institute of Food and Drug Safety Evaluation (NIFDS), Ministry of Food and Drug Safety, South Korea

*Woojung Lee is a research microbiologist. She has been with the Korea Ministry of Food and Drug Safety (MFDS) since 2014, has been with Division of Food Microbiology, Department of Food Safety Evaluation, National Institute of Food and Drug Safety Evaluation (NIFDS), Ministry of Food and Drug Safety (MFDS). She received M.S. degrees from the Department of Biotechnology at Korea University, South Korea in 2005. She has worked as a scientific researcher at Korea Institute of Science and Technology (KIST), South Korea in 2005-2013. Her major research interest involves microbial genomics for comparative analysis of food-borne pathogens, analysis on bacterial community in food, study of genome sequence information for food safety, establishment and operation of food-borne pathogen genome network.*

### Identification of Probiotic Bacteria in Foods through Metagenomic Approach

Probiotics are defined as "live microorganisms, which when administered in adequate amounts, confer a health benefit on the host". One of the most significant groups of probiotic organisms is the lactic acid bacteria (LAB), commonly used in fermented dairy products.

Identifying microorganisms in probiotic products is an important issue for products quality control and public health. Furthermore, misidentification of the probiotic LAB species might cause several health issues to consumers. The official microbiological testing methods of Food Code specify that LAB-containing products are confirming the number of lactic acid bacteria, but those methods have had some limitation to identify exact species of bacteria. To solve this limitation, in recent years, Next-Generation Sequencing (NGS) approach has been developed and various metagenomics classification tools have appeared. In this study, we developed LAB classification pipeline that effectively reduces false-positive using mapping coverage.

In order to examine the LAB detection ability of the pipeline, we used simulation data and NCBI SRA data, which consist of LAB species approved in Ministry of Food and Drug Safety (MFDS). We also investigated whether the bacterial contents specified on product labels were identical to actual bacteria species in probiotic products using metagenomic approach based on next generation sequencing (NGS). Actual samples were sequenced by NGS system such as Ion S5 or MiSeq and analysed using LAB classification pipeline.

In conclusion, LAB classification pipeline based on data produced by NGS is powerful tools to analyze bacterial contents in probiotic products and may improve food quality control. ■



### Pimlapas LEEKITCHAROENPHON / National Food Institute, DTU, Denmark

*Researcher, PhD. my research expertise includes whole genome sequencing (WGS) and epidemiology, evolution in bacterial genomes and population structure of foodborne pathogens. I have extensive experience in applying WGS in food safety and public health protection with the main focus on antimicrobial resistance. Some of my current projects include WGS analysis within the EU Reference Laboratory for AMR, the EU Horizon 2020 COMPARE project on source attribution using machine learning, and the Novo Nordisk Foundation project on AMR. In addition, I facilitated and conducted international bioinformatics training courses in WGS data analysis including online course on WGS analysis in COURSERA.*

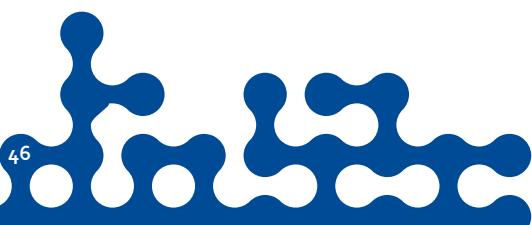
### Global surveillance of antimicrobial resistance through global sewage

Antimicrobial resistance (AMR) is a serious global public health threat and it threatens to undermine decades of progress in the treatment of infectious diseases. AMR is a complex problem with multiple and interconnected drivers. Current surveillance of AMR is patchy and mainly based on passive reporting of phenotypic laboratory results for specific pathogens isolated from human clinical infections.

From a surveillance point of view, urban sewage is attractive because it provides sampling material from a large and mostly healthy population, which otherwise would not be feasible to monitor. We characterized the bacterial resistome from untreated sewage from 79 sites in 60 countries across the globe in 2016. The sewage samples were sequenced on the HiSeq3000 platform (Illumina). The metagenomics sequences were mapped against resistance genes database (ResFinder) using MGMapper pipeline. Abundance of AMR genes was normalized into fragments per kilobase reference per million bacterial fragments (FPKM). There were systematic differences in abundance and diversity of AMR genes between Europe/North-America/Oceania and Africa/Asia/South-America.

The similar differences were observed in abundance of bacteria between Europe/North-America/Oceania and Africa/Asia/South-America. The association between antimicrobial use (AMU) and the abundance of AMR genes in the sewage samples was determined using national level of AMU data in 2015 from Europe ([www.ecdc.dk](http://www.ecdc.dk)) and IQVIA. The result showed that AMU only correlated with abundance of AMR genes in a specific antimicrobial class indicating that antimicrobial use data and bacterial taxonomy explained only a minor part of the AMR abundance and there are still other unexplainable factors that drive the abundance of AMR genes.

However, AMR abundance was strongly correlated with socio-economic, health and environmental factors, which we used to predict AMR abundances in all countries in the world. Improving sanitation and health could potentially limit the global burden of AMR. This study showed that sewage is a good source for an ethically acceptable and economically feasible continuous global surveillance and prediction of antimicrobial resistance and infectious diseases. ■



THURSDAY 28 MARCH

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# ROUND TABLE 1

## Handling multi countries foodborne outbreaks

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# FOODBORNE PATHOGENS & WHOLE GENOME SEQUENCING

JOINT CONFERENCE

## MODERATORS

### Charlotte GRASTILLEUR / ANSES, France

*Charlotte Grastilleur, originally graduated from Nantes Veterinary school in 1998 and specialized at the same time, from 1997 as a civil servant, in veterinary public health affairs related to food and feed safety and animal health, including animal welfare.*

*After completing her veterinary studies with a master thesis on dioxins in the food chain, she began her career in 1999 in local control services at the Direction départementale de l'agriculture de l'Oise (head deputy of veterinary services) for 3 years and had, as such, the opportunity to lead a team of 25 inspectors in all food safety sectors (retail, slaughterhouses, various industrial facilities benefiting from a European approval). She then joined the French central authority for food safety and animal health (direction générale de l'alimentation, general directorate for food at Ministry in charge of agriculture and food) in 2002: she started there as part of the national team for alerts/emerging risks and foodborne diseases clusters management (RASFF, listeriosis and salmonellosis...) and then moved to take on different positions, among which, head of the unit for general food legislation (bureau de la législation alimentaire) (chemical contaminants in food and improving agent, novel food and nanomaterial, radionuclides) for three years and head of the unit for seafood and freshwater products safety (bureau des produits de la mer et d'eau douce) for another three years.*

*She is now, from 2015 on, deputy head of the department for risk assessment at Anses, in charge of food-health related questions. These opportunities gave her the chance to get a comprehensive overview of major food safety challenges.*

### Hyo-Sun KWAK / National Institute of Food and Drug Safety Evaluation (NIFDS), Ministry of Food and Drug Safety, South Korea

*Dr. Kwak as a research microbiologist, she has been working for Korea Ministry of Food and Drug Safety(MFDS) for 27 years. She has been working in the area of risk assessment, methodology development, food standard establishment and act against foodborne disease outbreak in MFDS. She joined US Centers for Disease Control and Prevention(CDC) as a visiting scientist in 1995, and also Korea CDC for 1 year according to personnel interchange program in Korea.*

*Dr. KWAK launched the 'National Antimicrobial Resistance Management Program' in Korea in 2003, and continuously contributed to the reduction of antimicrobial resistance and use in Korea. In 2007, she worked actively for designating Korea as a hosting country of the Codex Task Force on antimicrobial resistance (AMR) and served as a secretariat of Codex TF AMR for 4 successful years. She also served as WHO expert group (AGISAR, Advisory Group of Integrated Surveillance of Antimicrobial Resistance) from 2014 to 2018, and participate in the ADB food safety management project from 2013 to 2017 in order to upgrade of the food safety level of LAO PDR. For the purpose of rapid reacting of foodborne outbreaks, she launched the MFDS mobile lab system, identifying disease causing agents within 4 hrs, in Korea for on-site outbreak investigation. This mobile lab has been used as a useful tool for prevention and control of foodborne diseases in international events such as 2018 Pyeongchang Winter Olympic games.*

*As a director of Division of Food Microbiology , she leaded the research group, 'Using NGS in foodborne pathogen management' since 2014 to build up genome DB of foodborne pathogens as well as strain bank for microbial pathogens isolated from outbreak in Korea. She also involved in research developing high-throughput data analysis software such as SNP pipeline, HGTree and Probiotics-analysis pipeline. Dr. Kwak is enthusiastic in building national DB for foodborne pathogens as well as improving quality of public health.*



## Stefano MORABITO / Istituto Superiore di Sanità (ISS), Italy

Director of the Unit of microbiological food safety and Food-borne diseases of the Department of Food Safety, Nutrition and Veterinary Public Health of the Istituto Superiore di Sanità in Rome, Italy. Director of the European Union Reference Laboratory for *E. coli*. He conducts researches on STEC and other pathogenic *E. coli* since 1996. As the Director of the EURL *E. coli* he coordinates the inter EURL Working group on NGS established by the EC in 2017. He is author of more than 70 peer-reviewed publications and edited the book "Pathogenic *Escherichia coli*, molecular and cellular microbiology", published by Caister Academic press.

## Martial PLANTADY / DG SANTE, European Commission, Belgium

Martial Plantady is a legislative officer working in the Directorate-General for Health and Food Safety of the European Commission. Graduated as a doctor in veterinary medicine with an additional Master degree in veterinary public health, he held different positions at the French Ministry of Agriculture from 1997 to 2006, dealing mainly with animal health issues, before joining the European Commission in 2007 where he is currently responsible for the control of zoonosis and antimicrobial resistance (AMR).

## Johanna TAKKINEN / European Centre for Disease Prevention and Control (ECDC), Sweden

Dr. Johanna Takkinen, DVM, MPH, joined European Centre for Disease Prevention and Control (ECDC) in 2005. She has a special degree in food- and environmental hygiene from the Faculty of Veterinary Medicine at the University of Helsinki in Finland and a Master of Public Health from the Nordic School of Public Health (NPH) in Sweden. Before joining ECDC, she worked for many years in official food laboratories as a laboratory veterinarian, leading the processes for accreditation in two laboratories between 1993 and 2002. While dedicated to improve quality of microbiological analytical methods, she worked as a technical assistant for the Finnish Accreditation Body for five years. After few years as a project researcher in the Finnish National Public Health Institute in 2002-2005, she joined ECDC as a senior expert in 2005. Dr. Takkinen has been coordinating the ECDC Food- and Waterborne Diseases and Zoonoses (FWD) programme since 2006. The programme covers surveillance and response to multi-country foodborne outbreaks of over 20 bacterial, viral, parasitic and prion diseases. The FWD programme is an umbrella for three European surveillance networks: Food- and waterborne diseases and zoonoses network (FWD-Net), European Legionnaires' disease surveillance network (ELDSNet) and European Creutzfeldt-Jakob Disease surveillance network (EuroCJD). Dr. Takkinen has a profound interest in understanding the molecular epidemiology of these diseases in EU/EEA and supporting the development of EU-wide surveillance and response to multi-country foodborne outbreaks by applying "One Health" approach.

## Henriette de VALK / Santé publique France, France

Dr. Henriette de Valk is a medical epidemiologist at the French National Public Health Agency (Santé Publique France). As the head of the Foodborne, Vectorborne and Zoonotic Infections Unit she is in charge of surveillance, outbreak investigations and applied research.



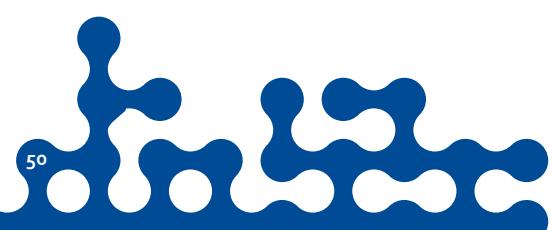
THURSDAY 28 MARCH

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## ROUND TABLE 2

# Data sharing and regulatory issues in the WGS era

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## MODERATORS

### Rene Sjøgren HENDRIKSEN / National Food Institute, DTU, Denmark

Dr. Rene S. Hendriksen is professor at the Technical University of Denmark, National Food Institute and act as director and deputy for the reference centres; World Health Organization Collaborating Centre (WHO CC) for Foodborne Pathogens and Genomics and the European Union Reference Laboratory in Antimicrobial Resistance (EURL AR), respectively. He provide advisory service to European Commission, European Food Safety Authority, WHO Global Foodborne Infection network, WHO Global Antimicrobial Resistance Surveillance System, WHO Advisory Group on Integrated Surveillance of Antimicrobial Resistance, Food and Agriculture Organization of the United Nations in the area of antimicrobial resistance and whole genome sequencing. His main focus is research in global epidemiology, surveillance, antimicrobial resistance, and population structure of mainly food and waterborne pathogens. He has been working with the most important food and waterborne pathogens including a long range of *Salmonella* serovars including *S. Typhi*, *Campylobacter* spp., *E. coli*, *Listeria monocytogenes*, *Shigella* spp., *Staphylococcus aureus*, *Klebsiella pneumonia*, *Vibrio cholera*, and *Enterococcus* spp.. Since 2010, the Research Group has embraced the new era of whole genome sequencing and lately also metagenomics with WHO and FAO supported initiate, "the Global Sewage Surveillance project" using a metagenomics approach to establish a global surveillance of human infectious diseases including antimicrobial resistance from sewage collected in major cities around the world to detect, control, prevent and predict human infectious diseases. Rene S. Hendriksen is author of 102 peer-reviewed published and accepted articles in international refereed journals conducted in collaboration with >500 scientists in >100 countries

### Karsten NÖCKLER / Federal Institute for Risk Assessment (BfR), Germany

Professor Nöckler studied veterinary medicine at the Humboldt University of Berlin, Germany and graduated in 1992. He is the head of the Department for Biological Safety at the German Federal Institute for Risk Assessment and has been working over 25 years on bacterial and parasitic foodborne zoonoses. He was involved in various national and EU research projects on zoonotic pathogens such as *Salmonella*, *Brucella* and *Trichinella*. Results of his scientific work were published in over 140 papers in peer reviewed journals.



# FOODBORNE PATHOGENS & WHOLE GENOME SEQUENCING

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## Marc ALLARD / Food and Drug Administration (FDA), Office of Regulatory Science, Division of Microbiology, USA

*Marc W. Allard is a Senior Biomedical Research Services Officer in the Division of Microbiology in FDA's Office of Regulatory Science. Dr. Allard joined The US FDA in 2008 where he uses Whole Genome Sequencing (WGS) of foodborne pathogens to identify and characterize outbreaks of bacterial strains, particularly *Salmonella*, *E. coli*, and *Listeria*. Dr. Allard specializes in both phylogenetic analysis, as well as the biochemical laboratory methods which generate the WGS information. Dr. Allard helped develop the first distributed network of laboratories that utilize whole genome sequencing for pathogen identification and traceback called the GenomeTrkr database, which is part of the NCBI Pathogen Detection web site. These tools are used daily for outbreak investigations. Dr. Allard acts as senior scientist to advise the US FDA on both WGS and phylogenetic methods as they apply to public health.*

## George HARINGHUIZEN / National Institute for Public Health and the Environment, the Netherlands

*George Haringhuizen (1954) studied Social Sciences (1983) and Law (2001). After a career as researcher and project leader in the field of welfare and refugees he entered the public health domain in 2001. Working at the Dutch Association of Public Health Services, George Haringhuizen was between 2001 and 2005 responsible for the taskforce to strengthen the national infrastructure for infectious disease control in The Netherlands. In 2006 he transferred to the newly established National Centre for Infectious Disease Control at RIVM, where he works as senior advisor to the director and as public health lawyer. He was responsible for the legal implementation of the International Health Regulations in The Netherlands and the Caribbean Overseas Territories. He was co-scribe of the revised Dutch Public Health Act (2008). George Haringhuizen published about law and infectious disease control in The Netherlands, European comparative public health law on influenza preparedness, and legal barriers for global WGS-data sharing. He acts as liaison to WHO on IHR, material- and data-sharing issues. He is actively involved as a legal advisor in the Global Microbial Identifier Network and currently leading a work package in the EU-funded COMPARE project, to develop an international platform and database for the rapidly sharing of WGS/NGS laboratory information for the detection and control of emerging infections and foodborn outbreaks. George Haringhuizen is guest-lecturer at the National School of Public and Occupational Health, at Erasmus University Rotterdam, and formerly at ECDC Stockholm (2015/2016).*

## Mirko ROSSI / European Food Safety Authority (EFSA), Italy

*Doctor in Veterinary Medicine with a PhD in Epidemiology and control of Zoonoses from University of Bologna, he received the title of Docent in Zoonotic Bacteriology from the University of Helsinki in 2015. From 2013 to 2018 he was appointed as associate professor at the University of Helsinki researching on genomic epidemiology of *Campylobacter* and other food-borne pathogens. Currently he is scientific officer at the European Food Safety Authority, Unit Biological Hazards and Contaminants.*

## Jørgen SCHLUNDT / Nanyang Technological University (NTU), Singapore

*Professor, Food Science at NTU. JS has worked nationally and internationally in research-based regulatory food safety 1983-99. Later Director Food Safety at the World Health Organization. and Director National Food Institute of Denmark. JS participated in international development of food safety Risk Analysis and the initiation of the Global Microbial Identifier, suggesting a global database of Whole Genome Sequences of all microorganisms.*



# List of Posters

# FOODBORNE PATHOGENS & WHOLE GENOME SEQUENCING

JOINT CONFERENCE

## THEME 1

### Microbiological surveillance and epidemiology

- ① Identification of persistent *Listeria monocytogenes* in food processing plants by using WGS can lead to modify cleaning and sanitation procedures

Salvatore ANTOCI

- ② Investigation of listeriosis outbreaks in small ruminants using pulsed-field gel electrophoresis and whole-genome sequencing

Bojan PAPIĆ

- ③ Managing and Analyzing NGS Data for Surveillance: a Hands-On Approach

Thomas HAVERKAMP

- ④ Assessment of persistence phenotype by *Listeria monocytogenes* Clonal Complexes Contaminating Seafood-Plants of Ready-To-Eat Products in France: Dynamics of Mobile Genetic Elements

Federica PALMA

- ⑤ Retrospective study of genomic epidemiosurveillance of *Salmonella* Dublin

Gaël PODEUR

- ⑥ WGS analysis of *Listeria monocytogenes* STg strains from meat processing facilities in Norway

Annette FAGERLUND

## THEME 2

### Foodborne pathogens characterization and outbreak investigation

- ① Advantage of whole genome sequencing in Staphylococcal Food Poisoning Outbreak

Déborah MERDA

- ② Bacterial Whole Genome Sequencing in Investigation of *Salmonella Typhimurium* Infection Outbreak, Riga, Latvia

Laima KIMSE

- ③ Characterisation of *Listeria monocytogenes* serogroup IIb isolated from meat products and meat food production environment

Monika KURPAS

- ④ Comparison of pulsed-field gel electrophoresis (PFGE) and whole-genome sequencing (WGS) for typing of *Campylobacter jejuni*

Darja KUŠAR

- ⑤ Genetic variety of food and veterinary isolates of *Listeria monocytogenes* in Latvia

Irena MEISTERE

- ⑥ Genome comparison of sorbitol-fermenting and non sorbitol-fermenting EHEC O157 – what's the difference?

Juliane DÜVEL

- ⑦ Outbreak of *Listeria monocytogenes* caused by consumption of raw fermented trout, Norway, 2018 to 2019

Lin T. BRANDAL

- ⑧ Performance evaluation of eight commercial DNA extraction kits for Whole Genome Sequencing for surveillance and outbreak detection: Shiga toxin-producing *Escherichia coli* (STEC) as a case study

Stéphanie NOUWS

- ⑨ Professional skills required to the "Food Microbial Bioinformatician"

Francesca POMPEI

- ⑩ The mobilome is a major contributor to *Escherichia coli* stx2-positive O26:H11 strains intra-serotype diversity

Sabine DELANNOY

- ⑪ The use of the Belgian molecular database and WGS-analyses in a multi-country outbreak investigation of *L. monocytogenes* ST6

Bavo VERHAEGEN

- ⑫ WGS analysis of *Salmonella* Welikade, a rarely described serovar involved in a foodborne outbreak in France in 2016

Emeline CHERCHAME

## THEME 3

### Quantitative risk assessment modelisation and source attribution in the genomic era

- 1 Unravelling the transmission of antimicrobial resistance between animals and humans with metagenomics

Ana Sofia RIBEIRO DUARTE

## THEME 4

### Pipelines and workflows for WGS data analysis

- 1 Development of a bioinformatics pipeline for routine analysis of whole genome sequencing data of *Escherichia coli* isolates

Bert BOGAERTS

- 2 Identification of recurring genotypes of *Campylobacter jejuni* within clinical isolates: new insights from a pilot study using three validated WGS-based typing schemes

Morgane NENNIG

- 3 Implementation and validation of pipelines for routine diagnostic based on sequencing data

Jörg LINDE

## THEME 5

### Antimicrobial resistance

- 1 A Curated Database of Complete Bacterial Plasmids

Pierre-Emmanuel DOUARRE

- 2 Are animals from the patients' farms a source of nosocomial LA-MRSA infection?

Jana AVBERŠEK

- 3 Characterization of *Serratia* species isolated from fresh vegetables using genomics

Gyu-Sung CHO

- 4 Genetic variety of plasmid mediated colistin resistance in Gram-negative bacteria from raw meat products

Alžběta BARÁKOVÁ

- 5 Global phylogenomics of multidrug resistant *Salmonella enterica* serotype Kentucky ST198

François-Xavier Weill

- 6 Imported foods as a transmission route of ESBL/pAmpC-producing Enterobacteriaceae

Paula OIKARAINEN

- 7 NGS-based workflow to improve the detection of antimicrobial resistance: from wet-lab to data analysis

Bas BERBERS

- 8 Presence of *Escherichia coli* strains producing extended spectrum β-lactamases (ESBL) in raw poultry meat from Greece

Economou VANGELIS

## THEME 6

### Metagenomics for food safety

- 1 Comparing metagenomics, WGS on isolates and routine classical microbiology methods for foodborne outbreak investigations with STEC as a case study

Florence BUYTAERS

- 2 Viral metagenomics of mussels from Slovenian coast

Urska JAMNIKAR-CIGLENECKI

## DEMO

Introduction of GenomeGraphR: A user-friendly open-source web application for foodborne pathogen whole genome sequencing data integration, analysis, and visualization

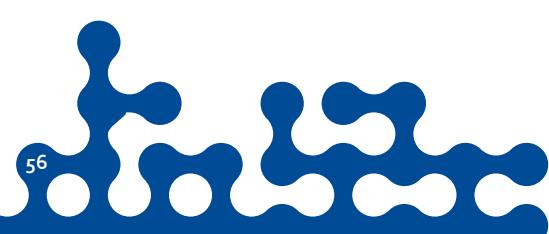
Moez SANAA



# FOODBORNE PATHOGENS & WHOLE GENOME SEQUENCING

## JOINT CONFERENCE

## NOTES



26-28 March 2019

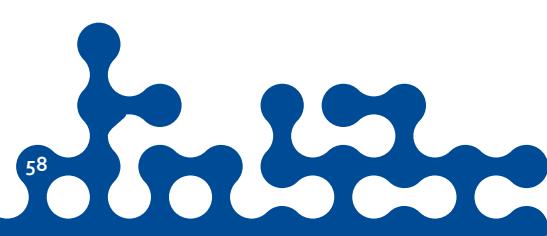
## NOTES



# FOODBORNE PATHOGENS & WHOLE GENOME SEQUENCING

## JOINT CONFERENCE

## NOTES



26-28 March 2019

## NOTES



# Foodborne pathogens & whole genome sequencing

## JOINT CONFERENCE



DTU Food  
National Food  
Institute



MINISTRY OF FOOD AND DRUG SAFETY  
National Institute  
of Food and Drug Safety Evaluation



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Yannick Blanchard, Laboratory of Ploufragan-Plouzané, ANSES  
Carlus Deneke, Molecular Microbiology and Genome Analysis, BfR  
Pimplapas Leekitcharoenphon (Shinny), Genomic epidemiology Group, DTU  
Burkhard Malorny, Molecular Microbiology and Genome Analysis, BfR  
Michel-Yves Mistou, Laboratory for food safety, ANSES, Head of scientific committee  
Soohwan Suh, Food microbiology division, NIFDS

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