Does WGS play a role for Food Safety?

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XII IMMEM, Dubrovnik
EFSA Molecular studies for Food Safety

- **Novel molecular biology methods**
- **Detection of emerging «high risk» clones**
- **Characterization/prediction resistance/virulence**
- **Novel computational approaches to predict drug metabolism and toxicity**
- **AMR surveillance (EUSR)**
- **GMOs**
- **Outbreak investigation (ROAs)**
- **Novel food**
- **Health claims**
- **Enzyme characterization**
- **Pesticides**
- **Food/Feed additives**
- **Guidelines applications regulated products**
- **Technical notes**
- **Genetic polymorphisms**
- **Effects chemical components on living organisms**
- **Source attribution**
- **Biomarkers**
- **Cloud**
- **Big data**
- **Transcriptomics**
- **Proteomics**
- **Metabolomics**
- **Metagenomics**
- **WGS**
- **OMICS**
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What is WGS for?

- **Species identification**
- **Strain characterization**
- **Plasmid characterization**
- **Resistance traits**
- **Persistence traits**
- **Pathogenicity, virulence**
- **Phylogeny**
  - Genetic relatedness

```
ACTGAACCTGTAGATCGTGAGATGTTTCTCCGGGCAACAGATTACAAAGTGACGGACCGTTGTACGTCCGCTGCTGAGATCGTTCCAGCTCCAGCGCTTTCTGATGCTGCGCCGCCGTTCCTGCTCAGATGTGCTCTATCACGTTCAGCCTTGTGACAACCGGCCTAGCTGCTCGCTCAAACTCTGCACCTGCCTGCTCAAGCCCGTGACGCTGCTCGCTCAGCGCCGCGTTCTCCCGTTGCATAAGACCAGACATCCTCGCATTCGCAAGGGCGGTCCCTACCTCCTAGCTTCTGCTGTAGCTGCTCTAATGCGGCAGCACTGTTTTTCCAGCTCCGTCATGTGCTATTTCCCCGCCAGTATCATCCAGCTCGAATCCTCTCGAAACGTCCCGCTTCCGGGTTCACCCTCACGCAGCGGCGTCTCTGCTCTCCGCAGGTCGAGAGCTGCACGCCGCTGTTCCTCTCGGACAGCATGGCCTGCGTGCTCTTCTGCTCCCGGATGATCGTGTAATTGTCGAGTATCTGCTGCCCCTGCCACCATAGAATGCTTGCGCCCGATGCTGTCAGCAGTGTAGACACCAGGACGATGGTCAGCCACGTCTGGCTGACCATACGCACCATGCCTTTCGTGCGCTGGCTCATGGCTGAGGACAGCTTCCGGTCG
```

"TAG the AGATA’s CAT tail*"

*Iolanda’s Mangone idea! (ISZ, Teramo, out bioinformatician for 1.5 years)
Foodborne pathogens and zoonotic bacteria
Q1. DO YOU CARRY OUT WGS ACTIVITIES? 28% YES (N=154 respondents)

- NRLs sequencing (17/30)
- Ols sequencing (3/14)
- NRLs non sequencing (13/30)
- Ols non sequencing (11/14)

Status December 2016

Current data collected by the EURs end 2017 show a similar picture (EC EURs WGS WG personal communication)
Q1...DO YOU CARRY OUT WGS... BY RESPONDENT (N=72 respondents)*

*72 respondents corresponding to a total of 183 replies from pathogen specific laboratories acting as NRLs. Analyses of data by “respondents” or by “Network laboratories” provided a similar picture for the whole questionnaire.
Examples of WGS for Food Safety

WGS for Outbreak investigations and other activities

Context: examples of work done somewhere else

Our reality: work done in/for EFSA
Do you remember? *E. coli* O104 Germany

May 2011

3816 cases

845 HUS cases

54 deaths

Photos kindly provided by Istvan Szabo,
National reference Laboratory for Salmonella,
Federal Institute for Risk Assessment, Berlin, Germany

Taken from Frank et al. NEJM 2011; 365:1771-1780
Prospective Genomic Characterization of the German Enterohemorrhagic *Escherichia coli* O104:H4 Outbreak by Rapid Next Generation Sequencing Technology

Alexandra Mollmann,1* Dag Harmsen2* Craig A. Cummings1* Emily B. Zenz1, Shana R. Leopold1, Alan Rico3, Karola Prior4, Rafael Szczepanowski1, Yongmei Ji5, Wenlan Zhang6, Stephen F. McLaughlin6, John K. Henkhaus5, Benjamin Leopold7, Martina Bielaszewska8, Rita Prager3, Pius M. Brzoska6, Richard L. Moore9, Simone Guenther9, Jonathan M. Rothberg9, Helge Karch1

1 Institute of Hygiene, University Münster, Münster, Germany; 2Department of Astrobiology, University Münster, Münster, Germany; 3LLC Technologies, Foster City, California, United States of America; 4Acumen, Gothenburg, Sweden; 5United States of America; 6LLC Technologies, Düsseldorf, Germany; 7Altair Koch Institute, Wiesendörfer, Germany; 8Proton Torrey Life Technologies, Guilford, Connecticut, United States of America; 9LLC Technologies, Berlin, Germany

Received June 29, 2011; Accepted July 6, 2011; Published July 20, 2011

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Open-Source Genomic Analysis of Shiga-Toxin–Producing *E. coli* O104:H4

Holger Rohde, M.D., Junjie Qin, Ph.D., Yunjun Cui, Ph.D., Dongfang Li, M.D., Nicholas J. Lumen, M.B.B.S., Moritz Hermsdörfer, M.D., Westong Chen, M.S., Fei Pu, B.S., Yanghong Peng, B.S., Jianhui Li, B.E., Feng Xu, B.E., Chengfeng Li, M.D., Xinxin Yan, M.S., Xiaojie Liu, M.S., Zhizhen Wang, B.S., Xiaoyan Zhang, M.S., Min Zhao, M.S., Peng Wang, B.M., Yuanfeng Guan, B.E., Zheng Cui, M.E., Xingyu Zhao, B.S., Martin Christen, M.D., Robin Kohn, M.D., Sebastian Loo, M.D., Jun Oh, M.D., Liang Yang, Ph.D., Antoine Darchen, Ph.D., George F. Cao, Ph.D., Bao Long Le, Ph.D., Yingwu Li, B.S., Xiaoming Yang, Ph.D., Guozhi Wang, Ph.D., Jianguo Xu, M.D., Ph.D., Mark J. Pallen, M.D., Ph.D., Jie Wang, Ph.D., Martin Applebacher, M.D., Rudolf Ting, M.D., and the *E. coli* O104:H4 Genome Analysis Consortium1


BGI, Uni Hamburg-Eppendorf

TY2482 (1/2. June)

Lifetech, Uni Münster

LB226692 (1/2. June)

Health Protection Agency

H112180280 (10 June)

www.hpa-bioinformatics.org.uk/lgp/genomes
Blogs and Wikis!!!!

**BacPathGenomics**
Genomics and evolution of bacterial pathogens

**Pathogens: Genes and Genomes**
A heady mix of bacterial pathogenomics, next-generation sequencing, type-III secretion, bioinformatics and evolution!

**CrowdSourcing**
Blogging the German outbreak E.coli 0104:H4

**GMO Pundit a.k.a. David Tribe**
Helping readers navigate the confusing myths of modern biology

EHEC Infection
@EHECInfection
· http://www.ehecinfection.com

Figures kindly provided by Kat Holt
London School of Hygiene & Tropical Medicine
Monash University, Melbourne

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DE isolates, little diversity, 2 SNPs in 4 isolates. FR isolates, 19 SNPs in 7 isolates. DE isolates formed a clade within the more diverse FR strains. Diversity present in the original set, and later reduced in the distribution to Germany?, or original homogeneous population subjected to different mutation rates upon their geographical segregation?
Detection methods: real time PCR (based on WGS data)
SCIENTIFIC REPORT OF EFSA

Shiga toxin-producing *E. coli* (STEC) O104:H4 2011 outbreaks in Europe: Taking Stock¹

European Food Safety Authority²,³

European Food Safety Authority (EFSA), Parma, Italy
Outbreak of *Listeria monocytogenes* in South Africa, 2017–2018: Laboratory Activities and Experiences Associated with Whole-Genome Sequencing Analysis of Isolates

Anthony M. Smith,1,2 Nomsa P. Tau,1 Shannon L. Smouse,1 Mushai Ailam,2 Arshad Ismail,2 Ntsieni R. Ramalwa,1 Boele Disenyeng,1 Mimmy Ngomane,1 and Juno Thomas1,2

>1000 cases, the largest *L. monocytogenes* outbreak

Listeriosis outbreak

- Mid-June 2017
- Several clinicians/microbiologists report unusually high numbers of listeriosis cases
- Signaled the start of the outbreak

>1000 cases, the largest *L. monocytogenes* outbreak

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Outbreak of *Listeria monocytogenes* in South Africa, 2017-2018

91% of human isolates are MLST sequence type 6 (ST6)

abcZ bglA cat dapE dat ldh lhkA

3 9 9 3 3 1 5

- WGS data
- minimum spanning tree
- drawn using cgMLST data (1748 genes)
- 695 isolates

Food, n=22
Human, n=328
Production facility, n=24

- WGS data
- maximum likelihood tree (linear tree)
- drawn using SNP profiles

Strain characterization

Trace back

Genetic relatedness

- ST6 cluster
- 91% of human isolates
- <10 SNP differences among isolates
- highly related

- Environmental ST6 isolates recovered from the food production facility
- ST6 isolates recovered from the company food samples

Grey shading links isolates showing ≤7 allele differences
- indicative of highly related isolates
- indicative of epidemiological relatedness

Figures kindly provided by Anthony Smith Centre for Enteric Diseases (CED), National Institute for Communicable Diseases (NICD) South Africa
Online tools and existing platforms: e.g. ENTEROBASE, BIGSdb, CGE, INNUENDO, COMPARE, PATRIC, etc.
Other EFSA activities for foodborne pathogens

“Technical support to collect and analyse whole genome sequencing (WGS) data in the joint ECDC-EFSA molecular typing database”

at least *L. monocytogenes*, *Salmonella*, *E.coli*

Published May 2019


**BIOHAZ PANEL**

“Self-tasking mandate for scientific opinion on the application and use of next generation sequencing (including whole genome sequencing) for risk assessment of foodborne microorganisms”

Deadline October 2019
WGS for AMR:
VIM-1 occurrence in the food chain

2011/2012
Positive farms (5)

2015/2016
Positive farms (1)

2017
Positive farms (3)

E. coli ST88 clone

Salmonella Infantis

Enterobacter cloacae

E. coli ST48

Salmonella Goldcoast

Salmonella Infantis

E. coli ST7593

pRH-R3 like IncHI2 plasmid

Roschanski et al. 2019. mSphere 4:e00089-19
SCIENTIFIC OPINION

Scientific Opinion on Carbapenem resistance in food animal ecosystems

EFSA Panel on Biological Hazards (BIOHAZ)

European Food Safety Authority (EFSA), Parma, Italy
Several human cases in the poultry associated clade reported direct contact with raw poultry meat

Larsen et al. (2016) – www.danmap.org
Joint scientific report of ECDC, EFSA and EMEA on meticillin resistant *Staphylococcus aureus* (MRSA) in livestock, companion animals and food.

Summary of the scientific Opinion of the Panel on Biological Hazards (EFSA/BIOHAZ) on “Assessment of the Public Health significance of meticillin resistant *Staphylococcus aureus* (MRSA) in animals and foods” and the Reflection paper of the Committee for Medicinal Products for Veterinary Use (EMEA/CVMP) on “MRSA in food producing and companion animals and in the European Union: Epidemiology and control options for human and animal health.”

Technical specifications on harmonised monitoring of antimicrobial resistance in zoonotic and indicator bacteria from food-producing animals and food

European Food Safety Authority (EFSA), Marc Aerts, Antonio Battisti, René Hendriksen, Isabelle Kempf, Christopher Teale*, Bernd-Alois Tenhagen, Kees Veldman, Dariusz Wasyl, Beatriz Guerra, Ernesto Liébana, Daniel Thomas-López and Pierre-Alexandre Beloel
Harmonized monitoring EU Legislation

Zoonotic Bacteria
- Salmonella spp.
- C. jejuni / C. coli
- ESBL-/AmpC-/CP-producing Salmonella

Indicator Bacteria
- E. coli
- E. faecalis / E. faecium
- ESBL-/AmpC-/CP-producing E. coli

Directive 2003/99/EC
Art. 7(3) and 9(1) + Annexes II (B) IV

Decision 2013/652/EU
2014 - 2020

New Decision
2021 - ...

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Selection of isolates:
- Emerging resistances
- Detection of clones
- Discrepancies

- Confirmation of results
- Ask MSs for the isolates
- Perform:
  - **WGS**, MIC re-testing
  - Seq. publicly available ENA

WGS analyses to support phenotypical AMR data: around 400 isolates selected sequenced by year
Sequences released to ENA (with permission of the MSs)
From WGS testing, detection of...

**Emerging AMR-Mechanisms?**

- **COL:** mcr-1, mcr1.6, mcr-3, mcr-4, mcr-5 ESBLs, PMQRs
- **AZT:** mefC-mphG tandem
- E. coli OXA-162

**Emerging AMR-Clones/Plasmids?**

- **S. Infantis ESBL in Italy**
- **S. Kentucky Cipro-R**

**Development of detection methods**

- Multi-plex PCR
Request for scientific and technical assistance on harmonised monitoring of antimicrobial resistance (AMR) in bacteria transmitted through food

Published June 2019


To address the use of molecular typing methods
- To complement and/or replace the phenotypic methods
- To ensure the comparability between the results of technics
- To integrate molecular data with past/future phenotypical data

...This includes the use of WGS
AMR tools? Benchmarking needed

Table 1. Results from Salmonella dataset for all antibiotic classes.

<table>
<thead>
<tr>
<th>Software</th>
<th>Specificity</th>
<th>Sensitivity</th>
<th>Accuracy</th>
<th>MCC</th>
</tr>
</thead>
<tbody>
<tr>
<td>KmerResistance</td>
<td>0.95</td>
<td>0.74</td>
<td>0.86</td>
<td>0.72</td>
</tr>
<tr>
<td>ResFinder</td>
<td>0.95</td>
<td>0.83</td>
<td>0.90</td>
<td>0.79</td>
</tr>
<tr>
<td>SRST2</td>
<td>0.93</td>
<td>0.80</td>
<td>0.87</td>
<td>0.74</td>
</tr>
<tr>
<td>PHE GeneFinder</td>
<td>0.97</td>
<td>0.83</td>
<td>0.90</td>
<td>0.81</td>
</tr>
</tbody>
</table>

Table 2. Results from E.coli dataset for all antibiotic classes

<table>
<thead>
<tr>
<th>Software</th>
<th>Specificity</th>
<th>Sensitivity</th>
<th>Accuracy</th>
<th>MCC</th>
</tr>
</thead>
<tbody>
<tr>
<td>KmerResistance</td>
<td>0.91</td>
<td>0.46</td>
<td>0.80</td>
<td>0.41</td>
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<tr>
<td>ResFinder</td>
<td>0.89</td>
<td>0.60</td>
<td>0.82</td>
<td>0.51</td>
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<tr>
<td>SRST2</td>
<td>0.89</td>
<td>0.57</td>
<td>0.81</td>
<td>0.48</td>
</tr>
<tr>
<td>PHE GeneFinder</td>
<td>0.90</td>
<td>0.53</td>
<td>0.81</td>
<td>0.47</td>
</tr>
</tbody>
</table>

Figure 1. Accuracy for three antibiotic classes from Salmonella dataset. Y-axis represents accuracy ratio expressed as a fraction of 1.

Figure 2. Accuracy for three antibiotic classes from E.coli dataset. Y-axis represents accuracy ratio expressed as a fraction of 1.

Taken from ENGAGE final mtg. 01.2018. Published Final Report

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Some tool give you all similar genes....
Aminoglycoside genes detection ......

<table>
<thead>
<tr>
<th>Sample name</th>
<th>File used</th>
<th>Trimmed</th>
<th>Assembler</th>
<th>Pipeline</th>
<th>aminoglycosides</th>
</tr>
</thead>
<tbody>
<tr>
<td>ERR2019166_Ena</td>
<td>ENA_reads</td>
<td>no</td>
<td>SPADES</td>
<td>A</td>
<td>aac(3)-Ila,aadA1,aadA2,aph(3')-la,strA,strB</td>
</tr>
<tr>
<td>ERR2019166_SRA</td>
<td>SRA_reads</td>
<td>no</td>
<td>Velvet</td>
<td>A</td>
<td>aac(3)-Ila,aadA1,aadA2,aph(3')-la,strA,strB</td>
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<tr>
<td>ERR2019166</td>
<td>ENA_assembly</td>
<td>yes</td>
<td>SPADES</td>
<td>B</td>
<td>AAC(3)-Ila,AAC(6')-Ib7,APH(3')-Ib (strA),APH(3')-la,APH(6)-Id (strB),aadA,aadA3,acrD,kdpE</td>
</tr>
<tr>
<td>ERR2019166</td>
<td>SRA_assembly</td>
<td>yes</td>
<td>SPADES</td>
<td>B</td>
<td>AAC(3)-Ila,AAC(6')-Ib7,APH(3')-Ib (strA),APH(3')-la,APH(6)-Id (strB),aadA,aadA3,acrD,kdpE</td>
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<td>no</td>
<td>C</td>
<td>C</td>
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<tr>
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<td>SPADES</td>
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<tr>
<td>ERR2019166</td>
<td>ENA_reads</td>
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<td>SPADES</td>
<td>D</td>
<td>AAC(3)-Ila,AAC(6')-Ib,AAC(6)-Ib7,APH(3)-la,APH(3)-lb,APH(6)-Id (strB),aadA,aadA15,aadA2,aadA22,aadA23,aadA24,aadA2_2,aadA8,aadA9,acrDchant(3)-la_1,aph(3)-la_1,aph(3)-lb_1,APH(6)-Id_1,kdpE,strA_1</td>
</tr>
<tr>
<td>ERR2019166T</td>
<td>ENA_assembly</td>
<td>yes</td>
<td>SPADES</td>
<td>D</td>
<td>AAC(3)-Ila,AAC(6')-Ib,AAC(6)-Ib7,APH(3)-la,APH(3)-lb,APH(6)-Id (strB),aadA,aadA15,aadA2,aadA22,aadA23,aadA24,aadA2_2,aadA8,aadA9,acrDchant(3)-la_1,aph(3)-la_1,aph(3)-lb_1,APH(6)-Id_1,kdpE,strA_1</td>
</tr>
</tbody>
</table>

Nomenclature chaos depending on the databases....
Proposed approach to integration of WGS ...
- Incremental approach over 2021-2026
- Voluntary use of WGS:
  - for Specific Monitoring of ESBL/AmpC/CP-producing E. coli (mandatory!)
  - For Confirmatory Testing using WGS

Harmonised protocol and quality criteria are needed!
- Several bioninformatic tools/pipelines/sequencing platforms
- Various reference AMR gene databases
- ... can hamper comparative accuracy of WGS results.

-\rightarrow EURL-AR to provide Harmonised Protocols/Quality Criteria and Training on DNA extraction, library preparation, sequencing in 2019-2020

Further developments in the future (re-assess regularly 2021-2026)
- ... To switch: EU-wide WGS data collection, leading to harmonized AMR mon.
- Provided a high concordance WGS- genotyping fand AST ... technology mature nd fully implemented ... and MSs ready.
Regulated products
Regulated products
WGS is useful for Food Safety...

• YES! A lot of information can be extracted from TAG, AGATA, CAT..

• We need to ask us always three questions
  • what do we want to know?
  • how can we achieve it?
  • which is the context?

• Different aims, similar/different bioinformatic tools needed.

• For surveillance/monitoring, harmonization is extremely important to allow comparability. The use of curated and updated data bases is a must.

• Detailed information on analysis done, tools, versions, parameters, etc. needed.

• Challenge: rapid and constant evolution of the techniques, tools, etc., can make reproducibility of the results difficult...

...reproducibility needs to be ensured
Thanks for your attention and thanks to...!

Ernesto Liébana, Valentina Rizzi, Pierre-Alexander Beloeil, Mirko Rossi, Elionora Sarmo, Montserrat Anguita, Jaime Aguilera, Nikoletta Papadopoulous (EFSA)
Iolanda Mangone (ISZ Teramo, EFSA Tasking Grant Bioinformatician)
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