Whole genome-based characterization of *Salmonella*, STEC, *Campylobacter jejuni* and *Listeria monocytogenes*

Aim:

Using WGS based methods for a) **elucidating *population structures, virulence factors and antimicrobial resistance genes*** in foodborne pathogens and b) ***tracing outbreaks***

1. Population structures, virulence factors and antimicrobial resistance genes

WP1.1: *Salmonella*

A novel lineage of ceftriaxone-resistant *Salmonella* Typhi from India that is closely related to XDR *S.* Typhi found in Pakistan

https://pubmed.ncbi.nlm.nih.gov/31872221/

Insight: Two MDR *Salmonella* Typhi isolates from India were found by whole genome sequencing to be closely related to the 2016 XDR S. Typhi outbreak strain from Pakistan.

Phylogenomic analysis of *Salmonella enterica* subsp. *enterica* Serovar Bovismorbificans from clinical and food samples

https://doi.org/10.3390/microorganisms10061199

Insight: The core genome analysis and the *k-mer*-binning methods characterized two polyphyletic clusters. These two polyphyletic clusters are two distinct genome pathotypes of *Salmonella* Bovismorbificans with emerging evolutionary properties.

Genomic analysis of *Salmonella enterica* Serovar Kentucky ST 198 strains isolated over 10 years from clinical cases in Switzerland

https://www.microbiologyresearch.org/content/journal/mgen/10.1099/mgen.0.000877#tab2

Insight: To understand the genomic diversity and antimicrobial resistance gene content associated with *S.* Kentucky in Switzerland, we whole-genome sequenced 70 human clinical isolates obtained between 2010 and 2020. Most isolates belonged to ST198-CIP[R]. High- and low-level ciprofloxacin resistance among CIP[R] isolates was associated with variable mutations in *ramR* and *acrB* in combination with stable mutations in quinolone-resistance determining regions (QRDRs). Analysis of isolates from patients with prolonged ST198 colonization indicated subclonal adaptions with the *ramR* locus as a mutational hotspot. SNP analyses identified multiple clusters of near-identical isolates, which were often associated with travel but included spatiotemporally linked isolates from Switzerland. The largest SNP cluster was associated with travellers returning from Indonesia, and investigation of global data linked >60 additional ST198 salmonellosis isolates to this cluster.

Salmonella Napoli
Project ongoing

WP1.2: Shigatoxin-producing *E. coli* (STEC)

Genetic characterization of Shiga toxin producing *Escherichia coli* belonging to the emerging hybrid pathotype O80:H2 isolated from humans 2010-2017 in Switzerland

https://www.sciencedirect.com/science/article/pii/S1438422118301887

Insight: All isolates carried *stx2a* or *stx2d*, the rare *eae* variant *eae*-ξ and at least seven virulence genes associated with pS88, a plasmid that is found in extraintestinal pathogenic *E. coli* (ExPEC). Core genome multi-locus sequence typing (cgMLST) detected two closely related but distinct subclusters with different *stx2* and *iuc*/*iutA* genotypes. All isolates were multidrug resistant (MDR). STEC/ExPEC hybrid pathotypes such as STEC O80:H2 represent a therapeutical challenge in the event of extraintestinal infection.

Characteristics of Shiga toxin-producing *Escherichia coli* isolated from raw meat-based diets for companion animals
https://doi.org/10.3390/microorganisms9081556

Insight: STECs were recovered from 41% of the 59 samples, and strains were subjected to serotyping and virulence gene profiling, using whole genome sequencing (WGS)-based methods. Of 28 strains, 29% carried stx2a or stx2d, which are linked to STEC with high pathogenic potential. Twenty different serotypes were identified, including STEC O26:H11, O91:H10, O91:H14, O145:H28, O146:H21, and O146:H28, which are within the most common non-O157 serogroups associated with human STEC-related illnesses worldwide.

Distribution of virulence factors, antimicrobial resistance genes and phylogenetic relatedness among Shiga toxin-producing *Escherichia* coli serogroup O91 strains from human infections 2003–2019 in Switzerland
https://doi.org/10.1016/j.ijmm.2021.151541

Insight: The strains belonged to O91:H10, O91:H14, and O91:H21. *stx1a* was the most common Shiga toxin gene subtype among the isolates, followed by *stx2b*, *stx2d* and *stx2a*. All strains were negative for the *eae* gene. STEC O91:H14 were distinguished from STEC O91:H10/H21 by one or more virulence factors found in extraintestinal pathogenic *E. coli* (ExPEC), including *hlyF*, *iucC/iutA*, *kpsE* and *traT* (hybrid STEC).

Whole genome sequence-based characterisation of Shiga toxin-producing *Escherichia coli* isolated from game meat originating from several European countries
https://www.nature.com/articles/s41598-023-30333-4

Insight: Game meat is becoming increasingly popular but may be contaminated with pathogenic bacteria such as Shiga toxin-producing *Escherichia coli* (STEC). In this study occurrence of STEC in 92 meat samples from chamois, red deer, roe deer, and wild boar, from Switzerland and other European countries was assessed. After enrichment, Shiga-toxin encoding genes (*stx*) were detected by PCR in 78 (84%) of the samples and STEC were isolated from 23 (25%) of the same samples. Nine different serotypes and eight different sequence types (STs) were found, with O146:H28 ST738 and O110:H31 ST812 predominating. None of the STEC belonged to the so-called top-five serogroups O26, O103, O111, O145, and O157. Subtyping of *stx* identified *stx1c*, *stx2a*, *stx2b*, *stx2e*, and *stx2g*. Additional virulence factors (VFs) comprised *ehx*, *iha*, *sta1*, and *subAB*. None of the isolates contained the *eae* gene. Twenty-one STEC contained VFs associated with extra-intestinal pathogenic *E. coli* (ExPEC).

WP1.3: *Campylobacter jejuni*

Whole genome-based characterization of *Campylobacter jejuni* from human patients with gastroenteritis collected over an 18-year period (2003-2020)
https://doi.org/10.1099/mgen.0.000941

Insight: We combined whole genome sequencing (WGS) and antimicrobial susceptibility testing (AST) of 340 randomly selected *Campylobacter jejuni* isolates from humans with gastroenteritis, collected in Switzerland over an 18-year period. The most common multi-locus sequence types (ST)s were ST-257 (n = 44), ST-21 (n = 36), and ST-50 (n = 35); the most common clonal complexes (CC)s were CC-21 (n = 102), CC-257 (n = 49), and CC-48 (n = 33). Source attribution based on ST assigned more than half of the strains to the "generalist" category (n = 188), 25% as "poultry specialist" (n = 83), and only a few to "ruminant specialist" (n = 11) or "wild bird" origin (n = 9). The isolates displayed an increased frequency of AMR from 2003 to 2020, with the highest rates of resistance observed for ciprofloxacin and nalidixic acid (49.8%), followed by tetracycline (36.9%). Quinolone resistant isolates carried chromosomal *gyrA* mutations T86I (99.4%) and T86A (0.6%), whereas tetracycline-resistant isolates carried *tet(O)* (79.8%) or mosaic *tetO/32/O* (20.2%) genes. A novel chromosomal cassette carrying several resistance genes, including *aph(3')-III, satA,* and *aad(6)*, and flanked by insertion sequence (IS) elements was detected in one isolate.

WP1.4: *Vibrio cholerae*

Genomic characteristics of clinical non-toxigenic *Vibrio cholerae* isolates in Switzerland: a cross-sectional study
Swiss Medical Weekly, accepted

Insight: In this study, we investigate the population structure and genetic characteristics of non-toxigenic *V. cholerae* isolates collected over 5 years from patients in Switzerland. Out of 33 *V. cholerae* infections reported between 2017 and 2022 in Switzerland, 31 were caused by *ctxA*-negative isolates. The 31 non-toxigenic isolates originated from gastrointestinal (n = 29) or extraintestinal (n = 2) sites. They were phylogenetically diverse and belonged to 29 distinct sequence types. Two isolates were allocated to the lineage L3b, a *ctxAB*-negative but *tcpA*-positive clade previously associated with regional outbreaks. The remaining 29 isolates fell into lineage L4, which is associated with environmental strains. Genes or mutations associated with reduced susceptibility to the first-line antibiotics fluoroquinolone and tetracycline were identified in 11 and 3 isolates, respectively. One isolate was predicted to be multi-drug resistant.

WP1.5: *Listeria monocytogenes*

Characteristics of *Listeria monocytogenes* from flowing surface waters in Switzerland
https://doi.org/10.1038/s41598-021-88514-y

Insight: The 25 isolates belonged to major lineages I and II, with the majority assigned to either serotype 1/2a (48%), or 4b (44%). The predominant CCs identified were the hypervirulent serotype 4b clones CC1 and CC4, and the serotype CC412; Two of the isolates belonged to CC6 which is an emerging hypervirulent clone. All isolates contained intact genes associated with invasion and infection, including *inlA/B* and *prfA*. The four CC4 isolates all harbored *Listeria* pathogenicity island 4 (LIPI-4), which confers hypervirulence

2.   Tracing (foodborne) outbreaks

Siblings with typhoid fever: an investigation of intrafamilial transmission, clonality, and antibiotic susceptibility
https://pubmed.ncbi.nlm.nih.gov/31580900/

Insight: This study investigated a family setting of severe typhoid fever in Switzerland months after return from Bangladesh. Four months after returning from a visit to Bangladesh sibling 1 (9 months) was diagnosed with a S. Typhi meningitis and sibling 3 (8 years) was identified as asymptomatic S. Typhi carrier. Sibling 2 (2 years) was retrospectively diagnosed with typhoid fever by IgM serology at the time point of admission to the hospital. Parents were asymptomatic and culture-negative. WGS analysis of family *S.* Typhi isolates showed clonality and strongest homology with *S.* Typhi strains occurring in Bangladesh. The *S.* Typhi strain showed resistance against fluoroquinolones. A 4-week course of ceftriaxone resulted in full recovery of sibling 1. *S.* Typhi was eradicated from sibling 3 following azithromycin treatment for 14 days.

Feedborne *Salmonella enterica* serovar Jerusalem outbreak in different organic poultry flocks in Switzerland and Italy linked to soya expeller
https://www.mdpi.com/2076-2607/9/7/1367

Insight: During 2020, seven *Salmonella* Jerusalem isolates from seven different poultry productions distributed over six cantons in Switzerland were reported, providing evidence of a possible outbreak. Using whole-genome sequencing (WGS*), S.* Jerusalem isolates from feed and from animals in Switzerland were further characterized and compared to *S.* Jerusalem from organic poultry farm environments in Italy. WGS results showed that feed isolates and isolates from Swiss and Italian poultry flocks belonged to the sequence type (ST)1028, grouped in a very tight cluster, and were closely related. This outbreak highlights the risk of spreading *Salmonella* by feed and emphasizes the need for a heat-treatment process for feed, also in organic poultry production.

Listeriosis caused by persistence of *Listeria monocytogenes* serotype 4b Sequence Type 6 in cheese production environment
https://pubmed.ncbi.nlm.nih.gov/33350924/

Insight: A nationwide outbreak of human listeriosis in Switzerland was traced to persisting environmental contamination of a cheese dairy with *Listeria monocytogenes* serotype 4b, sequence type 6, cluster type 7488. Whole-genome sequencing was used to match clinical isolates to a cheese sample and to samples from numerous sites within the production environment.

Massive spread of OXA-48 Carbapenemase-producing Enterobacteriaceae in the environment of a Swiss companion animal clinic
https://pubmed.ncbi.nlm.nih.gov/35203816/

Insight: A total of 22 (11.0%) environmental specimen yielded CPE, 14 (7.0%) ESBL-E, and 7 (3.5%) MRS; MR *Staphylococcus aureus* were isolated from two (10.0%) hand swabs. The CPE isolates comprised *Escherichia coli*, *Klebsiella pneumoniae*, *Enterobacter hormaechei*, *Citrobacter braakii*, and *Serratia marcescens*. Whole genome sequencing revealed that all CPE carried closely related bla_{OXA-48} plasmids, suggesting a plasmidic spread within the clinic. The clinic exhibited major deficits in surface disinfection, hand hygiene infrastructure, and hand hygiene compliance. CPE were present in various areas, including those without patient contact. The study documented plasmidic dissemination of bla_{OXA-48} in a companion animal clinic with low IPC standards. This poses a worrisome threat to public health and highlights the need to foster IPC standards in veterinary clinics to prevent the spread of ARM into the community.

Large multi-country outbreak of invasive listeriosis by a novel genomic clade of *L. monocytogenes* genoserotype IIa linked to smoked rainbow trout, 2020-2021

https://pubmed.ncbi.nlm.nih.gov/37036341/

Insight: We report the WGS-based identification of a large multi-national listeriosis outbreak with 54 cases that affected Germany, Austria, Denmark, and Switzerland during 2020-2021. Clinical isolates formed a highly clonal cluster (called Ny9) after core genome multi locus sequence typing (cgMLST). Routine investigations of food samples identified *L. monocytogenes* isolates from smoked rainbow trout filets from a Danish producer grouping with the Ny9 cluster. Patient interviews confirmed consumption of rainbow trout as the most likely source of infection. The Ny9 cluster was caused by a MLST sequence type (ST) ST394 strain belonging to molecular serogroup IIa forming a distinct clade within molecular serogroup IIa strains and had not been detected in Germany or the other three affected countries before. Reconstruction of a closed Ny9 genome and phenotypic characterization of several virulence-associated traits of a representative Ny9 isolate showed that the outbreak strain had the same pathogenic potential as other serogroup IIa strains. Our report demonstrates that international food trade can cause multi-country outbreaks which necessitate cross-border outbreak collaboration.

Multi-country outbreak of monophasic *Salmonella* Typhimurium sequence type (ST) 34 linked to chocolate products – the Swiss situation

Presentation at the ANNUAL CONGRESS OF THE SWISS SOCIETY FOR MICROBIOLOGY 30th AUG-1st SEPT 2022, Lausanne

Insight: An extensive multi-country outbreak of multidrug resistant monophasic *Salmonella* Typhimurium infection was linked to chocolate products produced by a large multinational company. In Switzerland, the first laboratory confirmed case was registered on 16.01.2022.
By the end of April 2022, 41 cases (mean 4 years, 24 female, 17 male) with isolates belonging to cluster 1 (MLST ST34; cgMLST 10106, HC5_296366), 0-2 cgMLST allele differences to reference strains SRR17830210 and SRR18021617) were identified. Between 29.03.2022 and 7.04.2022, two cases (4 years, female) with isolates belonging to cluster 2 (MLST ST34; cgMLST 4132, HC5_298160), 1 cgMLST allele difference to reference strain SRR18488397 were identified.

Screening and whole genome sequencing during an outbreak of *Serratia marcescens* in a neonatal and pediatric ICU

Presentation at the ANNUAL CONGRESS 2023, Paediatrie Shweiz, Interlaken

Insight: Two cases of invasive infections with *S. marcescens* in infants in the ICU of the University Children's Hospital Zürich prompted an outbreak investigation with infection control measures and screening in neonatal and pediatric ICU patients. *S. marcescens* isolates were characterized by whole genome sequencing (WGS). Between July 2020 and December 2022, in 41 critically ill children (age 4 days–14 years, 83% <1 year) *S. marcescens* was detected. 11 patients (27%, median age 32 days) were infected and 30 patients (73%, median age 84 days) were found to be colonized. The infections consisted of septic shock (n=4), central venous catheter infection (1), meningitis (1), pleuritis (2), ventilator associated pneumonia (2) and urinary tract infection (1). Whole genome sequencing identified 4 different cgMLST (core genome mulitlocus sequence typing) based clusters of *S. marcescens*. Eight infected and 6 colonized patients belonged to cluster 1 (isolates with ≤ 8 cg allele differences) which occurred over a period of 15 months. One infected and one colonized infant belonged to cluster 2 and two colonized children to cluster 3 and 4 each. In 30 of 533 screened patients (5.6%) *S. marcescens* colonization was detected. 25 (61%) colonized patients had a negative screening on admission indicating hospital transmission. Outbreak measures including strict patient zone control, reinforcement of hand hygiene, and sensibilization of staff members were implemented from October 2020 in an interdisciplinary team approach. Since November 2020 and introduction of screening a further 31 cases were detected, prompting further escalation of infection control measures. Since February 20222 screening identified only individual *S. marcescens* genotypes not related to the outbreak.

Rapid detection of the source of a *Listeria monocytogenes* outbreak in Switzerland through routine interviewing of patients and whole genome sequencing

Swiss Medical Weekly, accepted

Insight: In total, *L. monocytogenes* serotype 4b sequence type 388 strains from 20 patients belonged based on whole genome sequencing and cgMLST analysis to an outbreak cluster (≤1 different alleles between neighboring isolates). The outbreak occurred in North-Eastern Switzerland and the Zurich area with two exceptions. The median age (interquartile range) of patients was 77.4 (67.2-82.5) years (range 58.1 – 89.7). Men and women were equally affected. Rolling analysis of the interview data revealed smoked trout products from a local producer as a suspected source of contamination, leading to on-site control on 19 July 2022 by the cantonal chemist team and closure of the respective facility and a national wide product recall on 21 July 2022 after positive testing of food products of this facility. Food and production environment isolates from the respective facility could be linked based on WGS data to the outbreak cluster.