

[1] Comparative genomics of 16 strains of *Leuconostoc carnosum* isolated from cooked ham

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Leuconostoc carnosum is a lactic acid bacterium often used in meat industry as bioprotective starter due to production of bacteriocins against *Listeria monocytogenes*. This work aims to conduct a genomic diversity analysis on the genomes of 16 strains of *Leuconostoc carnosum* isolated from cooked ham packaged in modified atmosphere, manufactured by different European producers. These samples were isolated during the product shelf-life for a previous work and taxonomically characterized through 16S rRNA sequencing. For this work, their genomes have been analysed through whole genome sequencing, genes have been annotated and clustered into functional categories using RAST. Their pangenome was calculated with Roary. WGS analysis revealed that 4 strains belong to a different *Leuconostoc* species, *L. mesenteroides*. The pangenome of twelve *L. carnosum* strains consist of 2810 total genes; 1407 represent the core genome, 871 the shell genome, 532 the cloud genome. In order to identify bacteriocin-producing strains effective against *L. monocytogenes*, both on plate tests and *in silico* analysis using BAGEL4 were conducted. On plate test allowed to identify six strains, two *L. carnosum* and four *L. mesenteroides*. *In silico* analysis confirmed the results from plates, and it also identified another *L. carnosum* strain that possess the gene for bacteriocin but lacks the gene for a bacteriocin transmembrane transporter. This work is still in progress and its goal is to provide a better comprehension of *L. carnosum* genomics.

[2] PulseNet USA Transition to Whole Genome Sequencing as Gold Standard Method for Foodborne Disease Surveillance in the United States

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PulseNet, the national molecular subtyping network for foodborne disease surveillance in the United States, has transitioned in 2019 from using the previous gold standard of Pulsed Field Gel-Electrophoresis (PFGE) to Whole Genome Sequencing (WGS) as its primary subtyping method. PulseNet is a network of over 80 state, local, and federal public health laboratories that perform molecular subtyping on foodborne bacteria. PulseNet CDC has developed an analysis workflow including core and whole genome multilocus sequence typing (cg/wgMLST), genotyping tools to identify serotyping, virulence, plasmid, and resistance genes, as well as tools including an allele code built on single linkage trees to name each sequence for outbreak detection and easy communication. These tools and schema are integrated from open source tools available through the Center of Genomic Epidemiology (CGE), PubMLST, Institut Pasteur, and Enterobase and all sequence data generated by the states is uploaded in real time to NCBI and included in the GenomeTrakr databases. To access these tools, PulseNet members must participate in a certification and proficiency testing process that certifies that members can perform the WGS lab workflow to generate sequence data for all PulseNet organisms to meet quality standards and to accurately analyze the sequence data to determine whether it can be uploaded to a national database as well as NCBI. So far, over 50 PulseNet members have become laboratory and analysis certified, over 100,000 read sets for PulseNet organisms including *Salmonella*, *Escherichia*, *Vibrio*, *Listeria*, *Yersinia*, and *Campylobacter* has been uploaded to the PulseNet national databases and NCBI.

[3] Nanopore Whole Genome Sequencing for One-health: Identification of Pathogens, Antibiotic Resistance, and Virulence Factors

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Nanopore sequencing is a flexible alternative to public health in-depth characterization of pathogen genomes, and their antimicrobial resistance and virulence factors. The long reads have the potential to assemble the entire genomes, even from complex metagenomics datasets. We are collaborating with reference groups in microbiology, and in human and animal health from a One-Health approach, to validate the use of nanopore sequencing from isolates, from clinical samples (clinical metagenomics) or even from complex metagenomics samples. As a proof-of-concept, we have sequenced with MinION (1) 18 strains of *Staphylococcus pseudintermedius* isolated from pyoderma in dogs, and (2) 18 *Escherichia coli* positive for *mcr-1* isolated from a mixed farm (13 from calves, 4 from pigs and 1 from human). Unicycler was used to perform genome assembly and Abricate, along with different databases (CARD, NCBI and PlasmidFinder), to characterize the contigs. Nanopore sequencing allowed us to obtain the whole genome sequences of (1) the 18 *S. pseudintermedius* strains and to confirm their phenotypic resistance profiles at the genomic level; and (2) to identify the presence of colistin resistance due to *mcr-1* gene in 17 out of 18 *E.coli* strains and to locate it in the chromosome (2 strains) or at different replicon-harboring contigs (potential plasmids): IncX4 (14), IncI2 (1), and IncHI2 (1).

The lower accuracy of nanopore reads is overcome using a hybrid approach (e.g., Unicycler from Wick et al., 2017) with both long (nanopore) and short (Illumina) reads for the best assembly completeness and accuracy.

[4] Microbiological Criteria and Food Safety

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Currently Tunisia don't have a regulatory text that describe the microbiological criteria and their limits and that constitute a legislative reference for inspectors and controllers. Knowing that the contamination of food with microbiological agents is a public health problem worldwide, most countries have shown a significant increase over the past decades in the incidence of diseases caused by the presence of microorganisms in foods, including pathogens such as *Salmonella* and *E.coli*. Diseases caused by food-borne pathogens are a major burden for consumers. As a result, the prevention and control of these diseases have become public health goals internationally. These objectives depended in part on the establishment of parameters such as microbiological criteria, which reflect the knowledge and experience of good hygienic practices (GHP) during production, processing, handling, distribution, storage, sale, preparation and use, in conjunction with the implementation of the HACCP system. Microbiological criteria have been used for many years and have contributed to the improvement of food hygiene. It is within this framework that this project is integrated with the aim of producing a regulatory text setting the microbiological criteria and their limits applicable to all foodstuffs.

[5] Empowering local to global WGS-based surveillance and investigation: The Integrated Rapid Infectious Disease Analysis (IRIDA) Platform

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Specialized analytical tools and highly trained personnel are needed to analyze complex genomics data. Yet these resources are not always available in public health organizations. While centralized web services are available, these require users to upload potentially sensitive data to un-vetted servers, an action prohibited by some institutions and jurisdictions. The IRIDA platform is a decentralized, user-friendly, open-source bioinformatics and analytical web platform custom-designed to support multi-jurisdictional infectious disease outbreak investigations using genomic sequence data. IRIDA can be installed on institutional servers, enabling users to perform secure and local analyses, while also permitting data sharing with 'trusted' partners and public sequence repositories. IRIDA provides data management, secure data sharing, analytics and visualizations, and incorporates quality control, genomics assembly and annotation, in silico serotyping, multi-locus sequence typing, and outbreak phylogenomics. The platform also directly incorporates results into visualizations for hypothesis generation during epidemiological investigations. IRIDA supports customization through its new plugin architecture - connecting users to additional 3rd party tools, such as the Resistance Gene Identifier with its state-of-the-art AMR detection methods for genomes and metagenomes, as well as others in Galaxy Toolshed. Community development of IRIDA is encouraged and has led to customization success in Italy (ISS), and in South Africa to support LMIC (SANBI). Recent enhancements include preliminary ontology integration, and a data sharing ring trial. Future IRIDA augmentations include "containerizing" pipelines, cloud deployability, creating ontology-based fine-grained access controls, and developing a data attribution system. IRIDA is freely available at <https://github.com/phac-nml/irida> and www.irida.ca.

[6] Phenotypic and Genotypic Characterization of Antimicrobial Resistant *Escherichia coli* Isolated From Ready-to-eat Food in Singapore Using Disk Diffusion, Broth Microdilution and Whole Genome Sequencing Methods

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Antimicrobial resistance (AMR), especially multidrug-resistance, of bacteria is posing a great threat to public health. This study aimed to determine the antimicrobial resistance profiles of *Escherichia coli* isolated from ready-to-eat food sold in retail food premises in Singapore. In this study, a total of 99 *E. coli* isolates from poultry-based dishes (n=77) and fish-based dishes (n=22), obtained between 2009 and 2014, were included for disk diffusion testing. Of the 99 isolates, 24 (24.2%) were resistant to at least one antimicrobial agent. These isolates were then subjected to broth microdilution testing against 33 antimicrobial agents to determine the minimum inhibitory concentration (MIC) of isolates. Finally whole genome sequence (WGS) was carried out on the strains in order to correlate resistant phenotypes to putative antimicrobial-related genes. Of the 24 isolates, 15 (62.5%) were found to be resistant to three or more classes of antimicrobials and thus were defined as multi-drug resistant strains. Two isolates (8.3%) were confirmed as Extended-Spectrum β -lactamase (ESBL)- producing *E. coli* by double disk synergy test. Based on WGS data, online analysis tool ResFinder detected 7 classes of antimicrobial resistance genes and resistance-related chromosomal point mutations in 19 of the 24 *E. coli* isolates. By analyzing the WGS contigs using BLASTn and KmerFinder, ESBL genes and transferable colistin resistance gene *mcr-1* and *mcr-5* were determined to be located on plasmids, which could pose a greater risk of AMR transfer among bacteria. This study showed the presence of antimicrobial resistant *E. coli* isolates in ready-to-eat retail food, and raises a concern on the possible transmission of antimicrobial resistant bacteria from food to humans.

[7] Characterization of the Phenotypic and Genotypic Properties of Carbapenemase-producing *Vibrio* spp. Isolates in Germany

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Vibrio isolates are widely distributed in coastal waters and sometimes associated with wound infections and diarrheal diseases in humans. Some years ago, antimicrobial resistance testing of potentially pathogenic *Vibrio* species recovered from coastal waters of Germany indicated that some of the isolates exhibited carbapenem resistance. Recently, a *V. parahaemolyticus* isolate from imported Asian seafood intended for consumption in Germany exhibited also a non-wildtype phenotype against carbapenems. To determine the genetic basis of the carbapenemase-producing *Vibrio* spp., the isolates were subjected to whole genome sequencing and bioinformatical analysis. Sequence determination was performed by long- and short-read sequencing via PacBio RSII and MiSeq, respectively. Bioinformatic analysis revealed that carbapenem-resistant *V. cholerae* carried *bla*_{VCC-1}, while the *V. parahaemolyticus* isolate comprises *bla*_{NDM-1}. Further analyses, i.e. PFGE-profiling, DNA-hybridization as well as conventional PCR were used to reveal the organization of the *bla*_{VCC-1} or *bla*_{NDM-1} gene within the *Vibrio* genomes. Initial MiSeq sequencing of all prevailing isolates did not definitely revealed the genetic localisation of *bla*_{VCC-1} and *bla*_{NDM-1} within the genomes. However, PFGE profiling indicated that the *bla*_{VCC-1} resistance gene is chromosomally located, while *bla*_{NDM-1} is plasmidally encoded. Interestingly, some of the *bla*_{VCC-1} isolates carried more than one copy of the carbapenem-resistance gene on its chromosomes. The genetic basis of the *bla*_{VCC-1} and *bla*_{NDM-1} carrying genomes will be presented in detail. Our study indicates that carbapenemase-producing *Vibrio* spp. are frequently present in different regions of the German coastline and imported seafood. Therefore, the question arises if *Vibrio* species are a common reservoir for carbapenem resistance genes.

[8] Genetic variability and Antimicrobial Resistance of *Klebsiella* spp. Isolates from Sewage Water of Swine and Poultry Slaughterhouses

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Klebsiella spp. are Gram-negative opportunistic pathogens prevalent on plants, in water and soil but also colonizing a wide range of livestock/wildlife animals. Klebsiellae were recognized as an important threat to global public health due to their high level of antimicrobial resistance, mainly associated with the presence of mobile genetic elements. In this study, ESBL-producing *Klebsiella* isolates from sewage water of poultry and swine slaughterhouses were characterized by whole genome sequencing and antimicrobial resistance testing. Antimicrobial susceptibility testing of *Klebsiella* spp. isolates was performed using broth microdilution following CLSI guidelines and EUCAST epidemiological cut-off values. Whole genome sequencing and bioinformatics were performed to reveal the genetic basis of the observed resistances and the diversity of the isolates in different processing stages of slaughtering. Bioinformatic analyses revealed that prevailing isolates represented a high genetic diversity in its MLST-type and virulence profile. Overall, the isolates exhibited many plasmid sequences, that carried various antimicrobial resistance genes. The majority of the isolates harbor *bla*_{SHV} genes causing its ESBL-phenotype. Some of them also comprise determinants involved in the development of a carbapenem-resistance phenotype. The genetic basis of the isolates, their antimicrobial resistances and the content of mobile genetic elements will be presented in detail. Our study confirms that characterized klebsiellae comprises diverse mobile genetic elements that may be important vectors for the transmission of antimicrobial resistances. Some of the plasmids are closely related to plasmids of clinical *Klebsiella* isolates from humans. However, up to now their impact on human health is unknown and needs to be assessed.

[9] Dissection of the Genetic Basis of *mcr-4/mcr-5* carrying *Escherichia coli* Isolates from Food and Livestock in Germany

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According to the recommendation of the WHO, colistin belongs to the highest priority critically important antibiotics, which should be used only to treat severe human infections caused by multidrug- and/or carbapenem-resistant Gram-negative bacteria. In 2017, Carattoli *et al.* and Borowiak *et al.* simultaneously reported on the identification of two novel mobilizable colistin resistance genes in *Salmonella enterica* serovar Typhimurium, designated *mcr-4* and *mcr-5*, respectively. In the National Reference Laboratory for Antimicrobial Resistances in Germany, *mcr-4* and *mcr-5* carrying *E. coli* isolates from the German national monitoring program for antimicrobial resistance in zoonotic agents from the food chain were subjected to whole genome sequencing for determination of its genetic basis.

Molecular detection and typing of mobile colistin resistance genes among colistin-resistant *E. coli* recovered between 2010 and 2017 was performed using multiplex PCR analysis according to Rebelo *et al.* (2018). For genetic characterization whole genome sequencing using an Illumina MiSeq-benchtop sequencer was conducted *in house*. Comprehensive bioinformatical analyses were performed to identify and characterize the localization of the *mcr*-genes within the genomes and the genetic variability of the isolates.

Out of 800 colistin-resistant *E. coli*, *mcr-4* and *mcr-5* was detected in 13 and three isolates, respectively. Molecular analyses, whole genome sequencing and bioinformatics revealed that two variants of *mcr-4* (*mcr-4.2* and *mcr-4.3*) and *mcr-5* (*mcr-5* and *mcr-5.2*) are prevalent in German *E. coli* isolates. Overall, the isolates differ in their MLST-, sero- and fim-type but carry highly conserved *mcr-4* and *mcr-5* plasmid prototypes that showed some variability in size and genetic composition. Detailed information on the genetic features of the isolates and *mcr*-carrying plasmids will be summarized.

Our findings indicate that the mobile colistin resistance genes *mcr-4* and *mcr-5* are located on closely related plasmids that are non-self-transmissible. However, both *mcr*-genes are located in transposable elements that might be disseminated by transposition to other mobile genetic elements. Up to now, the impact of these resistance genes is unknown. Further information on the stability of *mcr-4/mcr-5* harboring genetic elements, their transmission routes as well as their distribution in livestock, food products and humans are needed to assess the potential impact of this resistance determinant on public health.

[10] Dynamic Time Warping Assessment and Sensitive High Resolution Melting Analysis for Subtyping *Salmonella* Isolates from Northern Thailand

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Nontyphoidal *Salmonella* spp. transmitted through various routes are a major concern of food poisoning due to the consumption of contaminated food. To establish a molecular-based protocol for simple and rapid subtyping of *Salmonella* isolates from various sources. Sensitive High-Resolution Melting-curve analysis (S-HRMa) and Dynamic Time Warping assessment (DTW) were applied for serotyping forty *Salmonella* spp. isolates from various origins and locations in seven provinces in the north of Thailand; the results were compared to those from conventional serotyping and ERIC-PCR. HRM serotyping of 40 *Salmonella* spp. initially produced 14 melting-curves with 2 predominant clusters: C1 (n=18) and C2 (n=9). Applying S-HRMa and serogroups generated 25 sensitive clusters. Conventional serotyping revealed that cluster C1 and C2 comprised of 6 different *Salmonella* serotypes with *S. Weltevredent* (n=14) as the predominant one. The S-HRMa also suggested the possible subtyping in some serotypes. In addition, DTW was performed to cluster those 40 *Salmonella* spp. into 28 clusters, assigned into different 4 clades corresponding to S-HRMa. The two clustering methods indicated that the *S. Weltevreden* was the predominant subtype (DTW4-S1, n=6). Three ERIC clusters at 92% similarity index also corresponded to the results of those two clustering methods. With important and related epidemiological data, *S. Derby* and *S. Monophasic* were suggested to be related to the slaughterhouse and swine. In this study, the ERIC cluster 10 comprising 2 *Salmonella* isolates of *S. Weltevreden* suggested the transmission route was likely to be farm-to-farm in the same province. The DTW assessment and S-HRMa effectively increased the discriminatory power of clustering to the same level as that of ERIC-PCR and were a simple and rapid protocol to perform *Salmonella* subtyping for the epidemiological research.

[11] Implementation of Next Generation Sequencing for Pathogen Typing in Routine Analysis - Evaluation of DNA Fragmentation Methods

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Next Generation Sequencing (NGS) currently represents the highest-resolution method for strain typing. In combination with a suitable data analysis, NGS has the potential to differentiate bacterial isolates of a species much more precisely than with the typing methods previously used in routine. The implementation of NGS in routine analysis, as part of the official monitoring of food, requires an optimization of the corresponding workflow with regard to sequencing quality and time/cost ratio. A crucial factor in NGS applications is the choice of method for DNA fragmentation used to produce DNA libraries. The prerequisite for a high coverage of the genome is the generation of random and uniform strand breaks. Coverage can vary depending on the type of fragmentation method selected and the DNA sequence of the organism. In order to evaluate the susceptibility of different methods to the sequence-induced generation of irregular fragment sizes, their application to bacterial DNA with different GC contents (*Campylobacter coli* with ~30 % GC content and *Salmonella enterica* with ~50 % GC content) was tested and evaluated in terms of time, cost and size distribution. Fragmentation was performed physically by ultrasonic on the one hand, and enzymatically by tagmentation or two alternative enzymatic methods on the other hand. The generated fragments were separated by capillary electrophoresis and the size distributions of the individual isolates were compared before and after size selection. When enzymatic kits were used, the DNA could be overfragmented due to sequence-specific fragmentation, while physical fragmentation is easier to standardize due to less fragmentation bias.

[12] High Throughput Whole Genome sequencing for Molecular Surveillance using a Certified Process

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Whole genome sequencing with Next Generation Technology (NGS) is a wide-spread method that is used in many laboratories as a “gold standard” for genome analysis. There are several methods available that can be used for the NGS library preparation, as well as for the sequencing procedure. Due to the different methods and instruments the generated sequencing data can differ in their composition and quality. As a result, different laboratories compare different data and may generate different analysis results. To use NGS also for high throughput surveillance studies without the loss of quality we set up an automated and time- independent full walk-away process. This process was certified with DIN ISO 17025 in the last year and is the basis to generate comparable sequencing data of the same quality for any kind of species.

[13] Challenges and Opportunities for Establishing High Throughput Sequencing at the National Veterinary Research Institute, Poland

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High Throughput Sequencing (HTS) technologies have been rapidly developed over the last decade proving their universal usefulness in genetic-based studies. Its most prominent application is whole genome sequencing (WGS) in outbreak and epidemiological surveys. Although outsourcing might be convenient, in-house sequencing capacity is needed for large scale studies, precisely in reference laboratories such as NVRI. Herewith milestones in setting up operational HTS laboratory are presented. Being a partner in the international ENGAGE¹ project (2016-2018; www.engage-europe.eu) gave a base for hands-on trainings, proficiency testing and gaining personnel competences. ENGAGE was also an incentive for the Ministry of Science and Higher Education (MoS) to grant resources² for equipment purchase for the newly created Department of Omics Analyses (2017). Usefulness of the implemented WGS was proved during the real-life scenario of multinational Salmonella Enteritidis outbreak related to Polish eggs. Timely response and support for the Ministry of Agriculture helped to convince national authorities on the advantages of WGS-based studies in food safety and protection of public health. Laboratory capacities have been also used in research projects such as EFFORT³ (2014-2018, www.effort-against-amr.eu) or One Health EJP⁴ (2018-2022, www.onehealthjep.eu). Broad and effective usage of granted resources was the reason for further MoS donations to support the sustainability of special equipment (2018-2020)⁵ and extension of research activities (2018-2019)⁶. Herein we show the establishment of High Throughput Sequencing laboratory that is capable of high-level research and reference testing in support for relevant national and regional authorities.

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MoS donations: 4477/E-180/R/2016²; 4477/E-180/SPUB/2018/1⁵ 4477/E-180/S/2018-1⁶

[14] Whole Genome Sequencing – a Support Tool in *Salmonella* Reference Laboratory

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Salmonella reference laboratory (1) continuously faces diagnostic and epidemiological challenges. Whole genome sequencing (WGS) is a great tool that allows to meet and overcome some of them. Here we show some examples in which WGS analysis with open CGE bioinformatics tools helped to answer vital diagnostic questions. Serovars Enteritidis, Infantis, Kentucky, and monophasic Typhimurium currently prevail in, respectively, laying hens, broilers, turkey, and pigs in Poland. WGS-based identification of sequence type (respectively, ST11, ST32, ST198, and ST34) confirms tested strains as part of the ongoing outbreaks or epidemics. Besides routine epidemiological investigations WGS supports and improves serotyping. The method fails to identify autoagglutinating strains, whereas WGS easily recognises them as the most common serovars, such as Enteritidis, Infantis, monophasic Typhimurium, or less frequent or exotic ones: Derby ST39, Brandenburg ST65, or Llandoff ST2321. Missing antigens might disturb efficient serotyping, but *Salmonella* 6,7:r:- and *Salmonella* 6,7:r:1,- were classified as Infantis ST32, whereas O:18 was identified in *Salmonella* IIIb -:l,v:z. Simultaneously, the strain represented novel *dnfN* allele with closest match to ST1262. Serotyping of rare and exotic serovars can be confirmed (i.e. Corvallis, Mapo – undefined ST, Salford, *diarizonae* 50:z₅₂:z₅₃, 53:z₁₀:z₃₅, *houtenae* 48:g,z₅₁:). WGS clearly demonstrates imperfection of KWLM scheme: we suppose serovar Bardo (8:e,h:1,2) does not exist and the strains belong to serovars Newport (6,8:e,h:1,2) ST166. In-depth analyses of *fliC* and *fliB* genes indicates reversed structure of flagellar antigens in KWLM listed serovar *diarizonae* 50:z:z₅₂. It is concluded that WGS improves quality and reliability of reference testing and may lead to scientifically relevant conclusions.

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[15] Food 3D Printing for Personalized Nutrition Based on Gut Microbiome

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Personalized nutrition is on the rise in the food industry and people are increasingly more informed about nutrition and health. There is an increasing number of tracking devices and apps to track the level of activity and food intake over time to personalize diet and exercise.

Companies like GX Sciences and circle DNA offer DNA testing kits that take a cheek swab to determine nutritional requirement based on human DNA.

3D printing of food enables the precise deposition of the desired ratio and amounts of ingredients. This offers a way to customize every meal to every individual.

A restaurant, Sushi Singularity, is opening in Tokyo in 2020 that provides 3D printed meals personalized to the nutritional requirements of the guest. The nutritional profiling is done using urine sampling a day in advance.

We intend to use stool samples to analyse the gut microbiome using whole genome sequencing and develop customized 3D printed meals accordingly.

The 3D printed meals would adapt to one's microbiome results in 3 ways:

1. Changing the amounts of various pre-biotics – For guts with all essential bacteria
2. Adding needed pro-biotics – For guts with missing good bacteria in people with otherwise healthy immune systems
3. Metabolites – For guts lacking the correct bacteria and compromised or underdeveloped immune systems (eg babies, patients with poor/damaged immune systems)

[16] Characterization of Extended Spectrum Beta-Lactamase-Producing *Escherichia coli* isolates from a reservoir in Singapore

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Extended Spectrum Beta-Lactamase-Producing *Escherichia coli* (*E. coli*) is increasingly detected from many sources including food, environment, clinic globally. The fast spreading of Extended Spectrum Beta-Lactamase-Producing bacteria and Extended Spectrum Beta-Lactamase (ESBL) genes has become a big challenge to public health. In this research, we characterized 9 ESBL-producing *E. coli* isolated from a reservoir in Singapore with both genotypic and phenotypic methods. Each of the cephalosporin resistant isolates is carrying at least 1 variant of *bla*_{CTX-M} gene. The most common beta-lactamase gene is *bla*_{CTX-M-15}. And all the isolates were shown to carry at least two transferable vectors. We use Next-Generation-Sequencing and online analysis tools to investigate the relationship between beta-lactamase and beta-lactam resistance. In this way, we are providing reference information for controlling and tracking beta-lactam resistance from environmental water source.