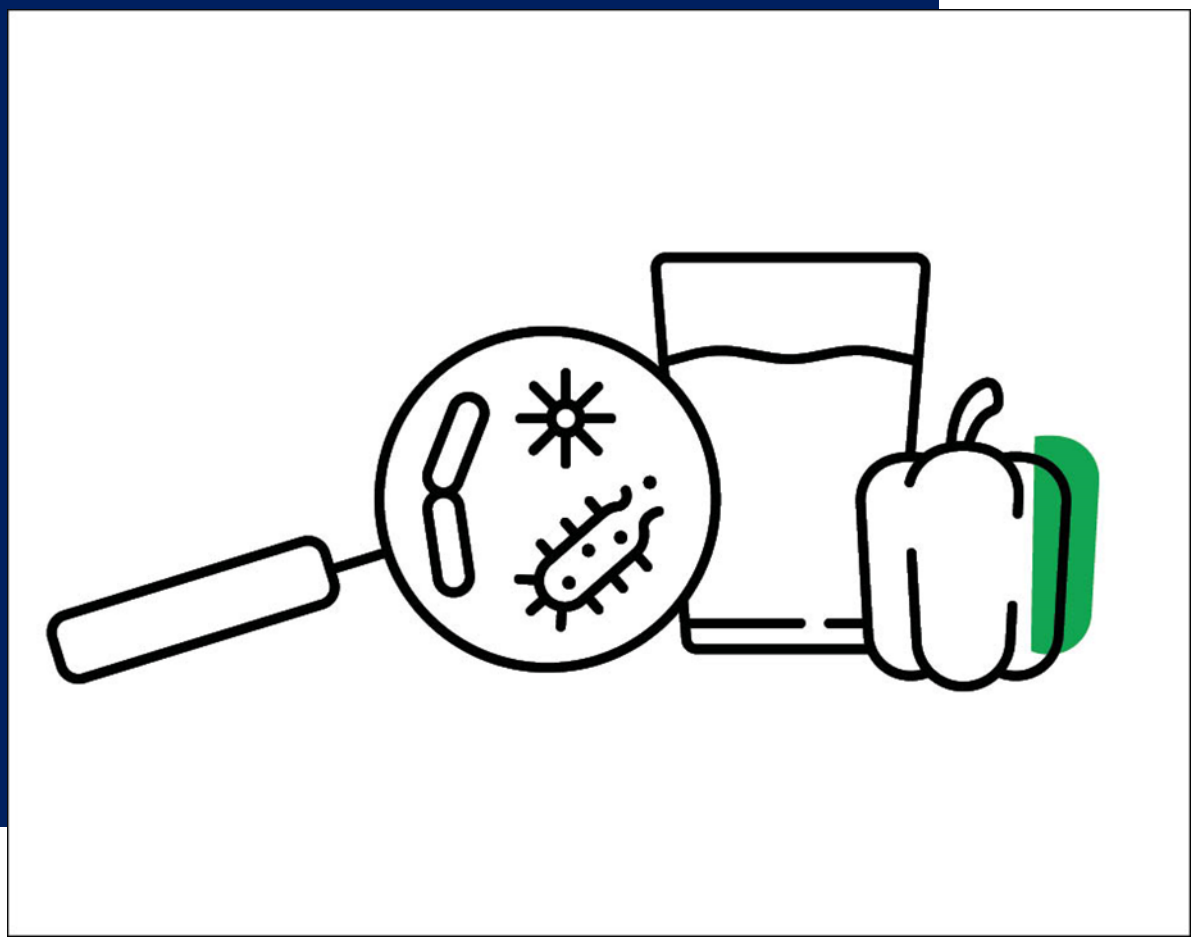
Abstracts



19. Conference of Food Microbiology

01–03 April 2025, Berlin

Vereinigung für Allgemeine und Angewandte Mikrobiologie

DGHM Deutsche Gesellschaft für Hygiene und Mikrobiologie

One Health Platform

BfR German Federal Institute for Risk Assessment

Preface

Dear Ladies and Gentlemen,

Welcome to the “19. Conference of Food Microbiology” at the German Federal Institute for Risk Assessment (BfR) in Berlin. This meeting is set up together with the special group “Food Microbiology and -hygiene” of the two German microbiological societies DGHM and VAAM and with the support of the One Health Platform.

The German Federal Institute for Risk Assessment is an independent institution of the Federal Ministry of Food and Agriculture (BMEL). It was set up in November 2002 to strengthen consumer health protection. The tasks of the BfR include the identification of hazards in food and feed and the risk assessment of food and feed. The results of its work serve as the basis for scientific advice to the relevant federal ministries and other agencies within Germany but also in international context.

One working area of the BfR lies in the field of food microbiology. Here, the BfR work is aimed towards pathogenic microorganisms in food and food production environment that pose a harm to the consumer. More than 100,000 cases of illness caused by various microorganisms in food (bacteria, viruses, parasites) are reported in Germany every year. This clearly indicates the need for research in this field. Questions about the occurrence of zoonotic pathogens along the food chain and measures to improve consumer health protection are increasingly linked to environmental aspects in the sense of the One Health approach and have to be answered in the future. To maintain and elevate the expertise of knowledge on distinct pathogens, the BfR harbours several national reference labs, consulting and special laboratories such as the NRL for *Listeria monocytogenes*, for Salmonella and for coagulase-positive Staphylococci incl. *Staphylococcus aureus*. The work of those laboratories is also driven by collaboration and knowledge sharing. This conference is a perfect opportunity for discussions and scientific exchange. The talks and posters that are going to be presented by scientists at different career levels, research topics and scientific perspectives will surely be inspiring and enlightening. We are happy that this conference is not only focussing on food pathogens but also on beneficial microbes. We are honoured that the “One Health Platform” supports this conference with one session.

With all the impressions and discussions ahead, I wish you all a wonderful “19. Conference of Food Microbiology” at the BfR.

Prof. Dr Dr Dr h. c. Andreas Hensel, President of the German Federal Institute for Risk Assessment

Ladies and gentlemen, esteemed colleagues, dear microbiologists,

On behalf of the VAAM and DGHM Special group food microbiology and hygiene it is our great pleasure to welcome you to the 19. Conference of Food Microbiology.

As a special group under the roof of the two German microbiological societies, the German Society of Hygiene and Microbiology (DGHM) and the Association of General and Applied Microbiology (VAAM), we aim to combine a variety of aspects regarding food microbiology. This was also our purpose for this conference held in the next days.

We are very happy to have presentations as talk or poster addressing the different aspects of food microbiology and hygiene. The topics of the presentations cover foodborne pathogens as well as beneficial microbes and microbiome research. Moreover, we are very lucky to have a session with the German One Health Platform helping us to broaden our view how microbes affect our food, our health and our environment.

After the years of “starvation”, we would like to see this conference as renewal of the conference series on food microbiology bringing together microbiologists from the fields of research, diagnostics and development. We encourage you to make use of the unique opportunity to discover and discuss with your colleagues and experts.

Dr André Göhler

Prof. Dr Rohtraud Pichner

Dr Christina Böhnlein

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17:15–17:30	**A microfluidic approach for characterization of whole-cell biosensors for bacteriocin detection** Niklas Fante, Bielefeld University, Germany
17:30–17:45	**Evaluating Digital PCR for Food Monitoring: A Case Study with *E. coli*** Ronny Kellner, QIAGEN GmbH, Hilden, Germany

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12:25–12:45	**Insights into the genome of *Lactococcus lactis* and *Lactococcus cremoris* starter culture** Melina Piesch, University of Hamburg, Germany
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11:00–11:20	**What happens in an aquaculture - stays in an aquaculture: Insight into the diversity and population dynamics of *V. parahaemolyticus* in a German biofloc system** Jens André Hammerl, BfR, Germany
11:20–11:35	***Arcobacter cryaerophilus* exhibits pathogenic potential in human colonic cell lines** Antonia Bachus, Freie Universität Berlin, Germany

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13:00–13:30	**Farewell**

2 Abstracts – oral presentations

2.1 Keynote: *E. coli* virulence genes: What matters and what doesn't?

Flemming Scheutz

The International Centre for Reference and Research on Escherichia and Klebsiella, Department of Bacteria, Parasites and Fungi, Statens Serum Institut, Kopenhagen, Denmark

To assist in the assessment of the clinical and public health risks associated with different STEC strains, STEC is classified into two main groups based on their clinical association with disease: HUSEC (HUS associated *E. coli*) have been shown to be closely associated with an increased risk of the severe complication Haemolytic Uraemic Syndrome (HUS). HUSEC is defined as positive for *stx2a* or *stx2d* usually in a background of an *eae* or *aggR* positive *E. coli*. All other STEC are considered "low-risk" STEC. This definition has been of considerable value in cases of human infection but is also problematic because not all STEC infections are fully characterized and coupled to reliable clinical information. Outbreaks with emerging hybrid strains continuously challenge our understanding of virulence potential and may result in incorrect classification of specific pathotypes such as the cross-over STEC-EAEC O104:H4 outbreak strain in Germany in 2011, which may deserve an alternative (sero)-pathotype designation.

The integration of mobile virulence factors in the stepwise and parallel evolution of pathogenic lineages of STEC collides with the requirements of a good taxonomy, which should separate elements of each group into subgroups that are mutually exclusive, unambiguous, and taken together, include all possibilities. The concept of (sero)-pathotypes is therefore challenged and the need to identify factors of STEC that absolutely predict the potential to cause human disease is obvious.

Two contradictory approaches in the detection and characterisation of *E. coli* in the past decade have increasingly and respectively broadened and limited our ability to assess the public health risks associated with different *E. coli* strains. On the one hand, whole genome sequencing (WGS) has revealed new combinations of relevant virulence genes that were traditionally associated with well-defined and separated pathotypes. On the other hand, the introduction of PCR detection in the primary clinical sector(s) has resulted in a decrease in the characterisation of clinical isolates. These techniques are often represented by commercial gastrointestinal panels which do not allow for characterisation of the new combinations, neither do they distinguish between the different species encoding the target virulence genes. Taken together, these two approaches threaten our ability to detect outbreaks and emerging pathotypes, maybe even new species with clinically relevant virulence genes. The presentation will use Danish surveillance data from the past 27 years to illustrate the challenges associated with the two approaches.

2.2 Keynote: Functional relevance of the gut microbiome – fact oder science fiction

Dirk Haller

Nutrition and Immunology, Technical University of Munich, Germany

Two decades ago, microbiome research was leveraged on the basis of next-generation sequencing, and the prospect of unravelling its functional impact on human health and disease susceptibility gains exciting significance in the development of clinical applications. The digestive tract is the most densely populated organ of the human body reaching an overall taxonomic diversity of more than 4600 different bacterial species with a functional capacity of estimated 170 million proteins. Interindividual variation of microbiota composition and the sparse functional understanding of microbiome-host circuits still hamper clinical application. In In order to understand the impact of diet in the context of microbiome-related interventions, I will present two human intervention trials to the role of probiotics in the regulation of early life colonization and exclusive enteral nutrition in the treatment of chronic intestinal inflammation.

2.3 Impact of primary production conditions on the microbiome of German bulk tank milk

Mareike Wenning[1,2], Annemarie Siebert[2], Gregor Fiedler[3], Hans-Georg Walte[3], Katharina Hofmann[2], Stefanie Gieschler[3], Genia Lücking[2], Christina Böhnlein[3], Charles M.A.P. Franz[3], Siegfried Scherer[2]

[1] Bayerisches Landesamt für Gesundheit und Lebensmittelsicherheit
[2] Technische Universität München
[3] Max Rubner-Institut Kiel

Raw milk gets contaminated with a large variety of microorganisms during the milking process, e.g. via the teats of the udder or the milking equipment. Not much is known, however, how and to which extent the different conditions during primary production influence the composition of the microbiome. It is therefore difficult to choose the right measure for beneficially influencing the microbiological quality of bulk tank milk.

The aim of this study was the comprehensive evaluation of the microbial community composition of raw milk from 350 farms throughout Germany and their farm specific influencing factors. 16S rRNA gene amplicon sequencing was performed to map the microbiome and information on conditions of housing, milking process and other factors were collected by questionnaires. The analysis focused in particular on the diversity of the microbiome, the genera responsible for the main variance and a correlation of production conditions with abundance of particular genera in the microbiome.

The raw milk microbiome is influenced by a multitude of factors, which is reflected by a very heterogeneous distribution of bacterial numbers and microbial diversity. Outliers were detected for each factor analysed, however, there were also correlations observed. An automated milking system was associated with an increased microbial count and organic production conditions with a significantly higher diversity and lower microbial counts as conventional production.

The large variance in the data, though, suggests that there are complex relations between factors and possible other determining factors not evaluated so far that may also impact the microbial composition. More studies focusing on a more specific and narrow choice of parameters are needed to better understand the interrelations of different farm managing practices in milk production.

2.4 Antimicrobial resistance of *E. coli* from fresh pig, broiler and turkey meat after 10 years of antimicrobial minimization concept in Germany

Bernd-Alois Tenhagen, Carolina Plaza-Rodriguez, Annemarie Kaesbohrer, Matthias Flor, Mirjam Grobbel

German Federal Institute for Risk Assessement (BfR), Unit Epidemiology, Zoonoses and Antimicrobial Resistance

In 2014, Germany implemented the antimicrobial minimization concept with the 16th amendment of the German medicinal products act. This study investigates whether this approach effectively reduced antimicrobial resistance (AMR) in *E. coli* found on fresh meat from pigs, broilers and turkeys.

Bacteria were collected from fresh meat samples as a part of a National Monitoring Program. Samples were collected at retail all over Germany, representing meat as purchased by consumers. *E. coli* were isolated by regional laboratories and sent to the BfR for antimicrobial susceptibility testing in accordance to the requirements of CID 2013/652/EU and (EU) 2020/1729.

Pork was sampled in odd years from 2015 to 2021 (except 2017). Broiler and turkey meat were sampled in even years from 2014 to 2022 (except turkey meat in 2020). A total of 1903 isolates was examined and included in the analysis. Fewer isolates were received from pork (210) than from broiler (948) and turkey meat (745). Over the whole period, the highest proportion of isolates with complete susceptibility (CS) was found in pork (64.3%), followed by broiler meat (21.1%) and turkey meat (19.2%). Multidrug-resistance (MDR) was most frequently detected in isolates from turkey meat (49.0%), followed by broiler meat (45.5%) and pork (16.2%). Over time, CS increased in isolates from pork and turkey meat, while MDR decreased in both. No change was observed in isolates from broiler meat. Resistance was consistently lowest in pork isolates. While in 2014, resistance was more frequent in isolates from turkey meat, it was more frequent in broiler meat in 2022.

Results indicate that the reduction of AMU in pigs and turkeys was associated with a decrease of AMR in meat of these species. However, resistance remained on a high level in broiler meat, which is in line with a failure to decrease AMU in broilers. Therefore, further efforts to reduce AMU are needed, especially in those sectors where to date no reduction has been observed.

2.5 Evaluation of FT-IR spectroscopy to discriminate Shiga-toxin-producing *Escherichia coli*

Miriam Cordovana[1], Lea Kirschner[1], Frederik Pankok[2], Ulrike Loderstaedt[2], Simone Scheithauer[2], Denise Dekker[3], Andreas Erich Zautner[4,5], Judith Overhoff[6], Miriam Werner[6], Andreas Wille[6], Ulrich Schotte[7], André Goehler[8], Hagen Frickmann[9,10]

[1] Bruker Daltonics GmbH & Co. KG, Bremen, Germany
[2] Department of Infection Control and Infectious Diseases, University Medical Center Göttingen, Georg August University Göttingen, Göttingen, Germany
[3] The One Health Bacteriology Group, Bernhard Nocht Institute for Tropical Medicine Hamburg, Hamburg, Germany
[4] Institute of Medical Microbiology and Hospital Hygiene, Medical Faculty, Otto-von-Guericke-University Magdeburg, Magdeburg, Germany
[5] CHaMP, Center for Health and Medical Prevention, Otto-von-Guericke-University Magdeburg, Magdeburg, Germany
[6] Institute for Hygiene and Environment, City of Hamburg, Hamburg, Germany
[7] Department of Animal Health and Zoonoses, Central Institute of the Bundeswehr Medical Service Kiel, Kronshagen, Germany
[8] Bundesinstitut für Risikobewertung (BfR), Berlin, Germany
[9] Department of Microbiology and Hospital Hygiene, Bundeswehr Hospital Hamburg, Hamburg, Germany
[10] Institute for Medical Microbiology, Virology and Hygiene, University Medicine Rostock, Rostock, Germany

Industry Abstract

Background. Shiga-toxin-producing *Escherichia coli* (STEC) strains are zoonotic and waterborne pathogens, which represent a serious thread for public health. They can cause severe diseases like haemorrhagic colitis and haemolytic-uremic syndrome (HUS), especially in children and in elderly people. In this study, we evaluated the discriminative power of Fourier-Transform Infrared (FT-IR) spectroscopy to distinguish STEC at serotype level.

Material/Methods. N=162 well characterized isolates (86 serotypes, different pathovars) were investigated. FT-IR analysis was performed by the IR Biotyper® system (IRBT - Bruker Daltonics, Bremen, Germany). The discriminatory power of IRBT was assessed by exploratory data analysis (HCA, PCA and LDA). First, a subset of 30 STEC isolates from the BfR collection (13 serotypes, 7 serogroups) were tested. Subsequently, these strains were merged with a previously investigated collection of strains (n=132). After that, a subset of n=6 STEC strains with unknown serotype were added, to simulate the application of IRBT in a prospective way.

Results. Exploratory data analysis performed on the first set of strains showed that IRBT clustering is correlated with the *E. coli* O serogroup. Moreover, within each serogroup, the different serotypes can be distinguished, and within each serotype, a correlation with the virulence factors was also observed. The same findings were confirmed when investigating the full dataset. The unknown stx-producing samples were classified by IRBT as belonging to serotypes O157:H7 (n=3), O91 (n=1), O26 (n=1) and O104 (n=1).

Conclusion. IR Biotyping showed the potential to classify STEC isolates at serotype level, demonstrating its potential suitability for infection control, public health and epidemiological studies. Further investigation including more strains are necessary to confirm and strengthen these promising preliminary results, and to assess its capability to discriminate different pathotypes.

2.6 A microfluidic approach for characterization of whole-cell biosensors for bacteriocin detection

Niklas Fante[1], Alexander Grünberger[2,3]

[1] Multiscale Bioengineering, Technical Faculty, Bielefeld University, Bielefeld, Germany
[2] Center for Biotechnology (CeBiTec), Bielefeld University, Bielefeld, Germany
[3] Institute of Process Engineering in Life Sciences: Microsystems in Bioprocess Engineering, Karlsruhe Institute of Technology, Karlsruhe, Germany

Food spoilage organisms and foodborne pathogens have long posed a threat to human well-being and continue to do so. With the rising number of multidrug-resistant pathogens, their relevance in the food and healthcare industries remains significant and could even increase in the future. To mitigate their potentially severe impact on society, novel compounds for food preservation and medical treatments, such as bacteriocins, are necessary. Whole cell biosensors can serve as powerful tools in screenings for rapid detection of novel bacteriocins, as well as for the direct identification and quantification of their antimicrobial activity and efficacy. However, it is essential to characterize these sensors to accurately understand and interpret the measured signals, particularly in relation to cell-to-cell signal heterogeneity and potential biological adaptation processes that could affect the sensor's output. Microfluidic systems offer precise control over defined environmental conditions, making them ideal for the systematic investigation and characterization of biosensors. Combined with live-cell imaging, microbial behavior can be analyzed with high spatio-temporal resolution to study the effects of novel bacteriocins on organisms at both the population and single-cell level. We present selected concepts for the microfluidic investigation of the behavior of the model organism L. innocua LMG2785 pNZpHin2Lm after exposure with the bacteriocin nisin and demonstrate their applicability for biosensor characterization. The results reveal single-cell dynamics and time-dependent cell-to-cell heterogeneity at different nisin concentrations, enabling detailed insights into the formation of subpopulations as well as the observation of possible nisin persistence.

2.7 Evaluating Digital PCR for Food Monitoring: A Case Study with *E. coli*

Miriam Hesse, Ronny Kellner, Dominic O`Neil, Christina Engel

QIAGEN GmbH, QIAGEN Strasse 1, 40724 Hilden, Germany

Industry Abstract

Effective food monitoring is essential to ensure food safety and prevent foodborne diseases. Traditional culture-based methods, while reliable, are slow and resource-intensive, making it difficult to keep pace with the growing diversity of foodborne pathogens. Molecular detection methods, such as digital PCR (dPCR), offer a faster and more efficient alternative. In this study, we evaluate the use of nanoplate-based dPCR for food monitoring, using *E. coli* as a showcase pathogen. Our test setup mimics the detection of Shigatoxin producing *E. coli* in enrichment cultures of minced meat samples spiked with *E. coli* cells. Time-series DNA extractions from enrichment cultures at 1-10 h after inoculation were performed using a newly developed kit designed to deplete animal DNA, improving the specificity of microbial detection. Based on our findings, we discuss potential future applications of dPCR in food monitoring, including the detection of toxin genes associated with specific microbial species, highlighting its advantages for rapid and precise food safety assessments.

2.8 Keynote: The role of bacterial dormancy in the food chain

Patrick Mikuni-Mester

Centre for Food Science and Veterinary Public Health, University of Veterinary Medicine Vienna, 1210 Wien

Effective monitoring of microbial pathogens is essential for a successful preventive food safety and hygiene strategy. However, as most monitoring strategies are growth-based, these tests fail to detect pathogenic bacteria that have entered dormancy states such as the viable but non-culturable (VBNC) state. Bacteria that enter the VBNC state lose their ability to grow on standard enrichment media, but remain metabolically active, show a drastically increased tolerance against antimicrobials and can remain infective or completely resuscitate from dormancy. While the ability of bacteria to enter the VBNC state has been known for several decades, their impact on public health and food safety has still been drastically under-researched. Since its first description in 1982, researchers have described the VBNC state for more than 100 different bacterial species, of which 67 are pathogenic bacteria and 35 are foodborne pathogens including *Campylobacter*, *Salmonella*, *Listeria*, *Escherichia*, *Staphylococcus*, *Bacillus* and *Vibrio*. Induction of the VBNC state is a response to either unfavorable environmental conditions, such as changes in temperature, nutrient or water availability, or potentially lethal stress by biocides, antibiotics or physical stress. Food is frequently exposed to such conditions during processing, transportation and storage providing ample opportunities for the induction of VBNC cells and dormancy states are linked to pathogens ability to persist in food production environments. In this context, an overview of the current knowledge as well as our own work regarding the following aspects of pathogens in the VBNC state will be presented:

- Analytical methods for detection and quantification of dormant bacteria
- Risk, occurrence and induction of dormancy in food production environments
- Inactivation/Disinfection of dormant bacteria

2.9 *Listeria monocytogenes* biofilms under conditions simulating the meat processing environment

Thalia Preuß[1,2], Stefanie Lüth[1], Sylvia Kleta[1], Thomas Alter[2], Sascha Al Dahouk[1,3], Szilvia Neuhaus[1]

1 German Federal Institute for Risk Assessment, Berlin, Germany
2 Freie Universität Berlin, Berlin, Germany
3 Robert Koch Institute, Berlin, Germany

Listeria monocytogenes (Lm) can persist in food processing environments for a long time, leading to recurring cross-contamination of foods. The biofilm lifestyle is considered to protect Lm, facilitating its survival even under harsh conditions. As previous studies often neglect the conditions prevailing in food processing facilities, we established an experimental biofilm model approximating the conditions in meat processing.

A screening of 33 Lm field isolates, originating largely from meat products, showed that the majority were weak biofilm formers. Non-motile isolates showed the lowest biofilm forming ability. Six isolates with different biofilm forming abilities were selected for further in-depth analysis. Only three of them showed an increase in the biofilm biomass over six days at 21 °C, indicating biofilm maturation over time. Next, the characteristics of biofilms formed at 21 °C, 12 °C (meat processing) and 4 °C (food storage) were analysed. Cell numbers (CFU) were influenced by temperature and incubation time, with the highest values at 21 °C after one day (five of six isolates). Interestingly, the CFU at day six tended to be higher at 12 °C (four of six isolates) and one isolate showed the highest CFU at 4 °C. Analysis of the biofilms by CLSM and SEM revealed mainly monolayers with microcolonies at 12 °C and 21 °C. Isolates differed in matrix production which in turn was influenced by temperature and incubation time. Different cell morphotypes were observed in addition to the typical rod form. For example, spherical cells were increasingly found at 21 °C and after six days of incubation. Striking was the chaining phenotype of one of the non-motile isolates, which could be explained by a mutation in the secA2 gene. When exposing Lm biofilms to benzalkonium chloride to investigate the effect of biofilm lifestyle on biocide susceptibility, a protective effect could be confirmed.

2.10 Investigation of *Listeria monocytogenes* colonizing lamb's lettuce

Tim Hoffmann, Agnes Weiss

University of Hamburg

Listeria monocytogenes is one of the most important foodborne pathogens because of the high hospitalization and mortality rate of people suffering from listeriosis (FDA). Little is known about how exactly *L. monocytogenes* attaches to vegetables, i.e., lamb's lettuce. Pathogenicity is highly strain dependent, giving rise to the question if there is a link between colonization and virulence. After contamination, the attachment and formation of biofilms on food poses a serious risk because it allows the bacteria to persist over long periods of time. Therefore, the aim of this study was to investigate the attachment and colonization to as well as the ability of *L. monocytogenes* to form biofilm on lamb's lettuce.

To study the attachment of *L. monocytogenes* on lamb's lettuce several strains isolated from plant foods as well as from clinical samples were characterized genotypically using WGS. To quantify the biofilm formation, crystal violet assays were performed. Fluorescently labelled strains of L. monocytogenes were generated. Confocal laser scanning microscopy (CLSM) of these strains, scanning electron microscopy (REM) as well as transmission electron microscopy were used to track attachment at the single cell level and "BiofilmQ" software was used for biofilm quantitative analysis.

Crystal violet assays showed that biofilm formation after 12-14 h was the highest. Fluorescently labelling *L. monocytogenes* using the chromosomally integrating plasmid vector pAD1 was successful and fluorescence microscopy was performed examining infected lamb's lettuce leaves. REM and CLSM imaging showed *L. monocytogenes* colonizing stomata of leaves and also biofilm and microcolony formation was observed on the surrounding areas. REM images suggest colonization of the stomata of lamb's lettuce could work as a protective measure against external influences and potentially pose a challenge for the food industry.

2.11 Sanitation of fresh-cut lettuce by plasma-treated water as innovative hygienic tool – broad band inactivation or specialized player on native food flora?

Uta Schnabel[1], Hauke Winter[2], Tim Urich[2], Jörg Ehlbeck[1]

[1] Leibniz Institute for Plasma Science and Technology, Felix-Hausdorff-Str. 2, 17489 Greifswald, Germany
[2] University of Greifswald, Institute for Microbiology, Felix-Hausdorff-Straße 8, 17489 Greifswald, Germany

Foods consumed raw, such as lettuce, can host food-borne human-pathogenic bacteria. In the worst-case, these diseases cause to death. To limit illness and industrial losses, one innovative sanitation method is non-thermal plasma, which offers an extremely efficient reduction of living microbial biomass.

To address the research question whether an application of plasma-treated water (PTW) in a lettuce washing process could interact with which microbial groups of the total viable count (TVC), different special agars were used to investigate its reduction efficiency on different native cultivable microorganisms. In combination with MALDI-ToF (matrix-assisted laser-desorption-ionization time-of-flight)-based determination the gained overview was diversified.

All tested cultivable microbial groups were reduced using PTW. Gram-negative bacteria showed a reduction of 3.81 log10, and Gram-positive bacteria of 3.49 log10, respectively. Additionally, fungi were reduced by 3.89 log10. These results were further validated using a live/dead assay.

MALDI-ToF mainly identified Pseudomonas spp. and groups of Bacillus on the tested lettuce. These results indicate that the PTW treatment could efficiently achieve a ubiquitous, spectrum-wide reduction of microbial life.

The PTW treatment inactivated a wide spectrum of the developed cultivable microbial spectrum on fresh-cut endive. These microbial reductions were dependent on the microbes' cultivability and known defense mechanisms (Gram-negative bacteria > molds/fungi > Gram-positive bacteria). The non- or less-selective inactivation ability of PTW was unexpected.

On the one hand, this could mean that it is difficult to separate plant-protecting commensals from plant and human pathogens. On the other hand, it indicates the broad application potential of the innovative plasma technology against microbial food spoilage.

2.12 Inactivation of *Listeria monocytogenes* and Listeria-specific bacteriophages by high hydrostatic pressure to evaluate the potential of combining high pressure and bacteriophages for meat products

Taniesha A. Setiadi[1], Ramona Nitzsche[1], W. M. C. Eshani Fernando[1], Kung W. Chiu[1], Heinz Moorkamp[1], Kemal Aganovic[1,2], Katharina Willenbücher[3], Frank Hille[3], Charles M. A. P. Franz[3], Christian Hertel[1]

[1] DIL Deutsches Institut für Lebensmitteltechnik e. V., German Institute of Food Technologies, Prof.-von-Klitzing-Str. 7, 49610 Quakenbrück, Germany
[2] Institute of Food Quality and Food Safety, University of Veterinary Medicine Hannover, Foundation, Bischofsholer Damm 15, 30173, Hannover, Germany
[3] Max Rubner-Institut, Federal Research Institute of Nutrition and Food, Department of Microbiology and Biotechnology, Hermann-Weigmann-Straße 1, 24103 Kiel, Germany

Introduction: *L. monocytogenes* is a ubiquitously occurring food-borne pathogen that can cause potentially life-threatening listeriosis. With its ability to survive and even grow under harsh conditions such as refrigerated temperature food producers must face the risk of contaminated ready-to-eat meat products. Combining high hydrostatic pressure and bacteriophages enables targeted inactivation of *L. monocytogenes* and decontamination of packaged products without the risk of recontamination and thus provides additional safety.

Objective: The influence of meat products and ingredients on the inactivation potential of *L. monocytogenes* by high hydrostatic pressure was determined. To evaluate the most effective treatment sequence, the susceptibility of bacteriophages to high pressure was also tested. Moreover, the effect of the treatment sequence on the inactivation of *L. monocytogenes* by the combination of high pressure and phages was investigated.

Methods: Two commercially available Listeria-specific bacteriophage products and an *L. monocytogenes* outbreak strain were subjected to high hydrostatic pressure in the presence of meat-related ingredients such as spices mixtures, sodium chloride and sodium nitrite, as well as on two ready-to-eat meat products, i.e. Lyoner, Salami. Phage application on the surface of meat products was done following the specifications of the manufacturer.

Results: In general, the bacteria were more resistant to high pressure than the phages. The ingredients and sausage matrices influenced the inactivation potential of both Listeria phages as well as of the *L. monocytogenes* strain. Although there were differences between the phage products studied regarding their susceptibility to high pressure, ingredients and meat products had a similar effect on their inactivation, i.e. either protective or supporting.

2.13 Selection and characterization of exopolysaccharide producing lactic acid bacteria for the stabilization of fruit preparations

Dor Zipori[1], Silvan Festini[2], Sybille Neidhart[2], Mario Jekle[2], Agnes Weiss[3], Herbert Schmidt[1]

[1] Department of Food Microbiology and Hygiene, Institute of Food Science and Biotechnology, University of Hohenheim, Garbenstrasse 28, 70599 Stuttgart, German
[2] Department of Plant-based Foods, Institute of Food Science and Biotechnology, University of Hohenheim, Garbenstrasse 25, 70599 Stuttgart, Germany
[3] Food Microbiology, Hamburg School of Food Science, University of Hamburg, Ohnhorststrasse 18, 22609 Hamburg, Germany

Fruit preparations, containing fruit pieces, which are stabilized in a fruit puree matrix by added hydrocolloids, are widely used in the food industry. Certain lactic acid bacteria (LAB) can produce exopolysaccharides (EPS), such as β-glucan, which act as natural stabilizers in fruit-based products.

This study employed phenotypic and genotypic screening to identify potential β-glucan-producing LAB strains. A total of 247 strains were analyzed for EPS formation on solid media, PCR detection of the gene encoding β-D-glucan-producing glycosyltransferase (gtf-2), and β-D-glucan capsule formation using specific antibodies. Six strains exhibiting both phenotypic and genotypic traits associated with β-glucan formation were selected for further characterization. Whole-genome sequencing and metabolic analysis revealed diverse metabolic capabilities, suggesting potential applications in fruit-based products and other food systems.

To evaluate their functionality in fruit purees, *Pediococcus parvulus* LTH 1110 and *Levilactobacillus brevis* TMW 1.2112 were selected for fermentation in peach and pear purees. Controlled fermentation experiments were conducted in peach puree for 72 h. The fermented purees were analyzed for viable cell counts, free sugars, and organic acid content. Freeze-dried fermentates were incorporated into model strawberry fruit preparations, which underwent rheological measurements. Results demonstrated a significant increase in viscosity for fruit preparations containing peach fermentate of *P. parvulus* LTH 1110 and *L. brevis* TMW 1.2112 compared to the unfermented controls.

These findings highlight the potential of β-glucan-producing LAB to enhance the rheological properties of fruit preparations, offering promising applications in innovative food product development.

2.14 Exploring the microstructure of water kefir grains

Pia Bethge[1], Yael Politi[2], Luise Ernst[3], Daniel Wefers[3], Florian Schattenberg[4], Susann Mueller[4], Thorsten Mascher[1]

[1] Chair of General Microbiology, Dresden University of Technology, Dresden, Germany
[2] B CUBE - Center for Molecular Bioengineering, Dresden University of Technology, Dresden, Germany
[3] Institute of Chemistry, Food Chemistry, Martin Luther University Halle-Wittenberg, Halle (Saale), Germany
[4] Department of Applied Microbial Ecology, Helmholtz Centre for Environmental Research-UFZ, Leipzig, Germany

Water kefir is a fermented beverage that is produced by adding water kefir grains to a sucrose solution with dried fruits. The fermentation takes 1-3 days, after which the grains can be reused for the next fermentation. The white to translucent grains have a typical size of 2-10 mm, an irregular shape and a gelatinous texture. They consist of a bacterial exopolysaccharide matrix and are primarily colonised by lactic acid bacteria, acetic acid bacteria and yeasts. It is known that the grain surface is covered by a mixed layer of these microorganisms. However, the internal structure of the water kefir grains remains unclear. Our aim was therefore to explore this in more detail and to map the inside of the grains.

To investigate their internal structure, whole and split grains were analysed using scanning electron microscopy (SEM). In addition, thin cross-sections of the grains were prepared and imaged with SEM and transmission electron microscopy (TEM).

The SEM and TEM images reveal that the grains generally consist of three distinct layers from the outside inwards: (i) a dense layer of microbial cells, (ii) a transition layer where the cells become increasingly embedded in the exopolysaccharide matrix, and (iii) the core composed of matrix and cell debris. Further experiments show that the macro- and micromorphology of the grains can be influenced by the cultivation medium. To better understand this phenomenon from both a chemical and microbial perspective, ongoing experiments focus on the structural characterization of the exopolysaccharide matrices using various analytical methods. Additionally, flow cytometry is used to analyse and compare microbial community patterns.

2.15 Insights into the genome of *Lactococcus lactis* and *Lactococcus cremoris* starter culture

Melina Piesch, Agnes Weiss

Food Microbiology, Hamburg School of Food Science, University of Hamburg, Ohnhorststrasse 18, 22609 Hamburg, Germany

Lactococcus lactis and *L. cremoris* strains are used as starter cultures in the manufacture of high-protein products. High-protein products often contain bitter peptides when they are obtained by first concentration and subsequent fermentation. The genes of the proteolytic system, especially prtp, are of particular interest here, as these may be associated with the presence of bitter peptides. In this project, two isolates from a starter culture were sequenced and compared.

The complete genome was sequenced using Illumina and Oxford Nanopore Technology, to obtain a zero-gap genome. Afterwards, the reads were assembled. The results show insights in the genome and plasmidome of each strain. Genomes of strains used as starter cultures contain plasmids with different sizes and gene clusters. The genomes were compared with known *L. lactis* and *L. cremoris* reference genomes, followed by bioinformatic analysis.

Analysis with Mauve software shows that almost 90 percent of the starter cultures genomes match the reference genomes. It is interesting to note that some genes are chromosomally encoded instead of plasmid-encoded or vary in their direction. For example, a Locally Collinear Block (LCB) of 34,579 base pairs of the chromosome of the *L. cremoris* strain is 66,700 bp downstream than in the reference strain *L. cremoris* MG1363. Furthermore, genes encoding a response regulator, which are plasmid encoded on the reference strain *L. lactis* 275, are part of the chromosomal DNA of the starter culture strain of *L. lactis*. Interestingly, the two prtp genes of the starter culture strains share 98% similarity according to ORthoANIu software. These findings can be used to answer further research questions about the genes encoding important enzymes and proteins of the proteolytic system and how they are responsible for the maintenance of bitter peptide levels in high protein products.

2.16 Keynote: Bushmeat and one health

Fabian Leendertz

University of Greifswald, Germany

Zoonotic diseases pose a major threat to human health, as demonstrated by outbreaks such as COVID-19, HIV, and the ongoing mpox (monkeypox) epidemic. One key risk factor is the human–wildlife interface, particularly in contexts involving intense contact through bushmeat hunting, trading, and consumption. In my talk, I will explore the complexity of this issue and present examples from sub-Saharan Africa.

2.17 Keynote: Consumption of raccoon meat - a new source of Trichinella infection?

Torsten Langner

Leipzig University, Germany

The raccoon (*Procyon lotor*) of the procyonid family occurs as an invasive species in Europe. The annual hunting bag in Germany exceeds 200.000 harvested individuals with increasing tendency. As carrier of several zoonotic pathogens, it is considered as species of public health concern (1). Due to the availability of the animals, there is an interest, to use the meat of the harvested animals for consumption. This poses a potential infection pathway for several pathogens for example the zoonotic parasite *Trichinella* spp. A critical control point of game meat, which could contain *Trichinella* larvae is the official trichinella investigation for which the predilection site is of harvested game is to be sampled. The data regarding the predilection site in raccoons is spare. The applicable EU regulation (2015 / 1375) does not indicate which part of the carcass has to be sampled.

To evaluate the epidemic situation of *Trichinella* spp. within the German raccoon population several muscels, that are discussed as a potential predilection site, were sampled from 992 individuals which were hunted between 2016 and 2021. These samples were investigated based on the artificial digestion method (2, 3). Next to one case of Trichinellosis a baffling case of nematode invasion into the muscles of one individual was detected. On hand *Trichinella* spp. was ruled out by morphology and molecular biological investigations, on the other hand these nematodes showed some typical characteristic of *Trichinella* spp. It was not possible to determine the species of these nematodes, so the potential zoonotic risk remains unclear.

The prevalence of *Trichinella* spp in raccoons seems to be lower in Germany than in the eastern neighbouring countries (4). However, available studies included mainly raccoons hunted in western parts of the country while the focus of *Trichinella* findings in boar (*Sus scrofa*) are focused in the eastern parts (5). So, the actual occurrence might be underestimated.

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2.18 Keynote: Microbiology of insects as food and feed

Nils Th. Grabowski, Amir Abdulmawjood, Madeleine Plötz

Institute for Food Quality and Food Safety (LMQS), Hannover University of Veterinary Medicine, Hannover, Germany

As with other natural taxa, the use of insects by mankind is a complex story. On one hand, there are species with a long and persistent history of domestication such as the honeybee (*Apis mellifera*) and the silkworm (*Bombyx mori*). Other insect groups have been used on a rather local level without major intents of domestication such as stinglees bees (Meliponini spp.) and cochineal (*Dactylopius coccus*) for honey and dye production, respectively. These few cases are backed by several thousand insect and other terrestrial arthropod species with a traditional use as foodstuff and in ethnomedicine. These traditions have developed by trial and error over the millennia and typically start with gathering them from the wild. Depending on the species and the cultural background, these traditions can either be widespread like the consumption of locusts (Acrididae spp.) or very local like the one of certain longhorn beetles (Cerambycidae spp.). More recently, some species have been farmed as food, feed or for medicinal uses ("productive insects").

Naturally, the micro and mycobiome of insects seems to be highly taxon-dependent, with a basic set of bacteria and fungi which seems to exist in all insects, plus subsets for the order, family, genus, and species. The final element of these biomes is apported by the environment in which the animals live in. Like in vertebrate animals, these biomes contain physiological, but also (potentially) pathogenic flora. Over the decades, most research on insect microbiology has been done with entomopathogens to control pests or to protect domesticated insects. With increasing awareness and consolidating farming, processing, and market placing practices, productive insect microbiology has come into the focus of public health veterinarians.

Samples of fresh and unprocessed insects usually have high bacterial counts (10^7 to 10^8 cfu/mg), with spore-forming bacilli and Enterobacteriaceae as most common findings in productive crickets, locusts, and mealworms, as well as coagulase-negative staphylococci, yeasts, and moulds. Salmonellae, *E. coli*, and listeriae usually do not occur in these samples unless animals were contaminated secondarily, e.g. by infected operators. However, *E. coli* can be found in productive flies, black soldier fly larvae (*Hermetia illucens*) because they are detritivorous. For the species certified as novel food insects – house cricket (*Acheta domesticus*), migratory locust (*Locusta migratoria*), and the yellow (*Tenebrio molitor*) and lesser mealworms (*Alphitobius diaperinus*) – microbiological specifications were published in the Union List. So far, these specifications are highly product-dependent and must be consulted thoroughly to evaluate laboratory findings.

2.19 Is there a spread of multidrug-resistant high-risk *K. pneumoniae* clones from food to humans?

Isidro García-Meniño[1], Jens Andre Hammerl[1], Azucena Mora[2,3,4]

[1] Department for Biological Safety, German Federal Institute for Risk Assessment, Berlin, Germany
[2] Laboratorio de Referencia de *Escherichia coli* (LREC), Departamento de Microbioloxía e Parasitoloxía, Facultade de Veterinaria, Universidade de Santiago de Compostela (USC), Lugo, Spain
[3] Instituto de Investigación Sanitaria de Santiago de Compostela (IDIS), Santiago, Spain
[4] Aquatic One Health Research Center (ARCUS), Universidade de Santiago de Compostela (USC), Santiago de Compostela, Spain

Background

Klebsiella pneumoniae (KP), especially the high-risk clones ST147 and ST307, can represent a public health threat as a major cause of nosocomial infections. However, comprehensive information on the impact of food on their dissemination from farm via food to human is lacking. This study aimed the detection and comparison of a collection of KP of human clinical origin and commercially meat products (poultry meat and pork) collected in the Northwest of Spain for evaluation of their zoonotic impact.

Material and Methods

KP isolates were subjected to in depth microbiological/molecular characterization including WGS analysis. In silico analysis was centred on the resistance/virulence gene content and the phylogenetic evaluation of the core genome regions. In addition, plasmid genomes were reconstructed from WGS and their transmission potential was evaluated by filter mating studies.

Results

The comparison of KP genomes revealed some overlaps between the different phylotypes from clinical and food products. WGS-based virulotyping confirmed that ESBL ST147/ST307 KP clones of meat products exhibited an substantial set of virulence genes of certain functional associations, which slightly differ from the spectrum of virulence factors of human isolates. Multidrug-resistant plasmids associated with acquired resistances against fluoroquinolones (qnr), 3rd 4th generation cephalosporins (blaCTX-M15) and high transmission rates were also detected, suggesting a linkage between the different compartments and/or a possible route of transmission.

Conclusions

Meat products may represent a yet underestimated source of clinically relevant KP related with human isolates of nosocomial infections. A deeper understanding of the zoonotic impact of these bacteria should be prioritized to i) prevent further spreading through the food chain and ii) the implementation of follow-up public health surveillance strategies.

2.20 High occurrence of *Staphylococcus aureus* in wild ungulates used for human consumption in Brandenburg, Germany

Tobias Lienen[1], Anneluise Mader[2], Maciej Durkalec[2], Martin H. Richter[1], Sven Maurischat[1]

[1] Department Biological Safety, German Federal Institute for Risk Assessment (BfR)
[2] Department Safety in the Food Chain, German Federal Institute for Risk Assessment (BfR)

Introduction: Staphylococcal food poisoning is a human health concern. *Staphylococcus* (S.) *aureus* may also cause human infections. In particular, the production of staphylococcal enterotoxin (SE), Panton-Valentine leukocidin (PVL), and toxic shock syndrome toxin (TSST) can lead to serious illnesses. This study aims to continuously monitor the occurrence of S. aureus in wild ungulates used for human consumption in Brandenburg, Germany.

Material and Methods: A total of 323 nasal swab samples were collected from wild boar, roe deer, fallow deer and red deer during the 2023/2024 hunting season. Samples were analyzed for *S. aureus* by selective enrichment. Species were determined using MALDI-TOF-MS. Whole-genome sequencing was performed for genotyping and analysis of virulence genes. The phylogenetic relationship of the isolates was analyzed using core genome multilocus sequence typing. Moreover, phenotypic antimicrobial resistance (AMR) was determined.

Results: *S. aureus* was recovered from 58 nasal swabs corresponding to a detection rate of about 18%. The highest detection rate was found in fallow deer (39%) followed by red deer (32%), roe deer (16%) and wild boar (10%). S. aureus isolates were associated with different sequence types (STs), including ST1, ST30, ST45, ST97, ST133, ST425, ST3224, ST3255, ST4090 and ST6238. Some isolates showed high clonality, although they were isolated from different sources. Genes encoding SE or SE-like (SEl) were found in 26 isolates. The TSST-encoding tsst-1 gene was detected in one isolate. No genes encoding PVL were found. Regarding AMR, most isolates (50/58) were susceptible. One-fold (7/58) and two-fold (1/58) resistance were occasionally found.

Conclusion: Wild ungulates may carry enterotoxigenic *S. aureus*. Food poisoning due to SE-producing strains may occur if recommended hygiene measures are not applied during processing of game meat. Moreover, safe handling of animals or their carcasses can mitigate the risk of infection.

2.21 Keynote: Microbiology and food history: Tasty and dangerous encounters

Michael Brauer

University of Salzburg, Austria

Microbiology is a vital component of nutrition, as microorganisms play a crucial role in various areas of food production, processing and safety. This lecture will explore the history of nutrition, with the aim of demonstrating that traditional cultures, which had no concept of microorganisms such as bacteria, were able to utilise their effects through observation and experimentation. Fermentation in particular has had a profound impact on making food more digestible and preservable (dairy products, bread, alcoholic drinks and sauerkraut). These methods, vital for survival, have contributed to the development of a remarkable diversity of flavours throughout history. Beer in Ancient Egypt, for example, was akin to liquid bread and was imbibed with a straw.

But how did people cope with unwanted microorganisms? What preservation methods existed before the invention of the fridge and the cold chain? And is it true that spices were used in large quantities in the Middle Ages to mask the flavour of spoiled meat? The second part of the lecture aims to dispel common misconceptions about food safety in the past and to provide a more objective perspective on the subject.

2.22 Keynote: Rohmilch aus SB-Automaten: Ready to drink?

Jan Kabisch[1], Stefanie Gieschler-Lübbehüsen[1], Jannike Loop[1], Mareike Wenning[2], Helene Oberreuter[3], Dominik Baaken[4], Sebastian Hess[4], Charles M. A. P. Franz[1], Christina Böhnlein[1]

[1] Max Rubner-Institut, Institut für Mikrobiologie und Biotechnologie, Hermann-Weigmann-Str. 1, 24103 Kiel
[2] Bayerisches Landesamt für Gesundheit und Lebensmittelsicherheit (LGL), Veterinärstraße 2, 85764 Oberschleißheim
[3] Chemisches und Veterinäruntersuchungsamt Stuttgart (CVUA-Stuttgart), Schaflandstraße 3/2, 70736 Fellbach
[4] Institut für Agrarpolitik und Landwirtschaftliche Marktlehre, Universität Hohenheim, Schwerzstraße 46, 70599 Stuttgart

Im letzten Jahrzehnt ist die Verbrauchernachfrage nach regionalen und möglichst unverarbeiteten Lebensmitteln stetig gestiegen. Gleichzeitig befinden sich die Erzeuger in einem schwierigen Marktumfeld mit kleiner werdenden Gewinnmargen und hartnäckigen Verhandlungen mit den Molkereien wieder. Hier bietet die Tierische Lebensmittel-Hygieneverordnung - Tier-LMHV unter § 17, Absatz 4 - Abgabe von Rohmilch oder Rohrahm an Verbraucher zum einen die Möglichkeit unverarbeitete Lebensmittel zu erwerben und zum anderen ein alternatives Einkommen zu erzielen. Rohmilchmilch ist jedoch nicht zum Rohverzehr bestimmt, da es sich durch das mögliche Vorhandensein von pathogenen Bakterien um ein Risikoprodukt handelt., Es muss daher zwingend am SB-Automaten auf die Notwendigkeit des Abkochens der Rohmilch hingewiesen werden, was jedoch von Verbraucherinnen und Verbrauchern oft nicht umgesetzt wird.

Ziel unserer Untersuchungen war es daher, den Status-quo hinsichtlich der mikrobiologischen Beschaffenheit der abgegebenen Rohmilch Deutschlandweit zu erfassen und Schwachstellen im Hygienemanagement beim Betrieb von Abgabeautomaten zu identifizieren.

In den Untersuchungen zeigte sich, dass in ca. 15% der Proben pathogene Bakterien nachgewiesen werden konnten. In zwei Proben wurden mit Listeria (L.) monocytogenes und Shigatoxin-bildende *Escherichia coli* (STEC) sowie L. monocytogenes und Yersinia enterocolitica O:9 jeweils zwei pathogene Keimgruppen isoliert.

Die Untersuchungen der Hygieneparameter zeigten ein diverses Bild: die Gesamtkeimzahl schwankte sehr stark zwischen 2,9 - 7,8 log KbE/ml, wobei ein Drittel der Proben oberhalb der Grenze für das zweimonatige Mittel von 5 log KbE/ml lag, das die VO (EG) Nr. 853/2004 für Anlieferungsmilch vorgibt. Die größte Streuung wiesen die Werte für Pseudomonaden auf, die teils unterhalb der Nachweisgrenze von 10 KbE/ml lagen, aber auch Maximalwerte von bis zu 6,6 log KbE/ml erreichten. Bei 37% der untersuchten Rohmilchproben wurden mehr als 4 log KbE/ml an Pseudomonaden nachgewiesen. Im weiteren Verlauf des Vortrages werden Risiken und Chancen der Direktvermarktung aus lebensmittelhygienischer Sicht beleuchtet.

2.23 „Zukunftslabor 2030“ – Analytik, künstliche Intelligenz und digitaler Zwilling - Fleischverderb am aktuellen Beispiel von Hackfleisch

Clara Wimmer, Mareike Wenning, Melanie Pavlovic, Ingrid Huber, Ulrich Busch

Bayerisches Landesamt für Gesundheit und Lebensmittelsicherheit (LGL), Oberschleißheim

Im Rahmen des Projektes Zukunftslabor 2030 (ZL2030 – Zukunftsszenarien für den Verbraucherschutz auf Basis von Qualitäts- und Sicherheitsinformationen von Lebensmitteln, innovativen Messmethoden und KI) soll mit Hilfe Künstlicher Intelligenz (KI) eine Prognose der sensorischen und mikrobiologischen Qualität ausgewählter Lebensmittel ermöglicht werden. Grundlage hierfür sind verschiedene Messverfahren, wie Next-Generation Sequencing (NGS), Spektroskopie oder Analysen des Volatiloms (flüchtiger Substanzen), mit denen ein digitaler Zwilling (DZ) des Produktes erstellt wird. Durch die Integration verschiedener Messmethoden sollen die wichtigsten chemischen, physikalischen und biologischen Prozesse von Lebensmitteln durch den DZ beschrieben werden.

Bei der Bewertung des Frischezustandes von Lebensmitteln müssen die gestiegenen Ansprüche des Verbrauchers an die Qualität mit den Bedürfnissen der Lebensmittelhersteller und des Handels in Einklang gebracht werden. Es handelt sich hier um einen der zentralen Punkte im Spannungsfeld zwischen Lebensmittelsicherheit und Lebensmittelverschwendung, denn die Frische von Lebensmitteln beeinflusst nicht nur ihre sensorischen Eigenschaften, sondern auch ihre Sicherheit und Haltbarkeit. Gerade bei frischen und sehr leicht verderblichen Waren wie Fleisch und Fleischprodukten können variierende Bedingungen entlang der Lebensmittelkette - von der Herstellung über den Transport und den Einzelhandel - die Produktqualität beeinflussen, noch bevor sie beim Verbraucher ankommen. Diese Beeinflussung der Produktqualität hängt dabei sowohl von dynamischen Veränderungen hinsichtlich physikochemischer Vorgänge als auch ihres Mikrobioms, also der Gesamtheit der im betreffenden Lebensmittel vorkommenden Mikroorganismen ab.

Analytischer Schwerpunkt des LGL im Rahmen dieses Projektes sind NGS-basierte Analysen insbesondere des Mikrobioms und dessen Veränderung über die Dauer einer Lagerung der Lebensmittel oder in Bezug auf unterschiedliche Lagerbedingungen (wie z.B. Temperatur oder Gasphase in der Verpackung). Im Fokus stehen zunächst leicht verderbliche und nicht fermentierte Lebensmittel wie z.B. Hackfleisch, bei denen mikrobiologischer Verderb ein relevanter Aspekt der Lebensmittelsicherheit ist. Die besondere Herausforderung bei der Analyse von Verderbserregern liegt hier in der vergleichsweise geringen bakteriellen Keimzahl im frischen Produkt und der großen Menge an eukaryotischer Begleit-DNA aus dem Fleisch.

Im Vortrag werden Ergebnisse der Mikrobiom-Analyse (Metabarcoding) mikrobieller Populationen an Messreihen von Hackfleisch vorgestellt.

2.24 Insights into the microbial community of lamb´s lettuce

Jennifer Griese, Agnes Weiss

Food Microbiology, Hamburg School of Food Science, University of Hamburg, Ohnhorststrasse 18, 22609 Hamburg, Germany

Leafy vegetables, such as fresh lamb's lettuce, gain increasing popularity with consumers. However, as leafy greens are usually eaten raw, a potential safety risk from pathogenic microorganisms is associated with their consumption. All plants are colonized by an autochthonous microbiota dominated by bacteria and fungi. The composition of the plant's microbial community depends on numerous determinants. Host factors such as the genotype and environmental factors like soil type or climate have a major influence in shaping the autochthonous plant microbiota. There are indications that the microbiota of plants will shift with the ongoing climate change.

In order to understand the influence of climate change, a comprehensive understanding of the microbial community of leafy greens is of key importance. Therefore, a total of 36 samples of lamb's lettuce from Germany, Italy, France, and Austria, grown under different cultivation conditions, were collected and analyzed using metagenomics to examine the microbial community, with a special focus on pathogens. The five most abundant organisms, in each case of fungi and bacteria, are represented by *Cladosporium, Itersonilia, Plectosphaerella, Sampaiozyma*, and *Dioszegia*, and by *Flavobacterium, Sphingomonas, Methylobacterium, Pseudomonas*, and *Duganella*. The most important food-borne pathogens associated with leafy vegetables, *Listeria monocytogenes*, *Bacillus cereus*, Shiga toxin-producing *E. coli* and *Salmonella* spp., were not detected by specific PCR. In addition to a contamination with pathogens, it is possible that plants actively recruit human pathogenic species from the soil due to their beneficial properties for the plant. *Pseudomonas aeruginosa*, the species of particular importance in this context, was also not detected by specific PCR. The investigation underlines the importance of understanding the microbiota of leafy vegetables in order to consume raw leafy vegetables as safe food.

2.25 What happens in an aquaculture - stays in an aquaculture: Insight into the diversity and population dynamics of *V. parahaemolyticus* in a German biofloc system

Claudia Jäckel, Diana Manta, Cornelia Göllner, Jonas Nekat, Jens Andre Hammerl

German Federal Institute for Risk Assessment, Department Biological Safety, Max-Dohrn Str. 8-10, 10589 Berlin, Germany

Vibrio spp. has the potential to exert a considerable influence on the field of aquaculture. While some species are pathogenic to fish and seafood, others, including *V. vulnificus, V. cholerae* and *V. parahaemolyticus*, have the potential to cause intestinal and extraintestinal infections in humans. In order to mitigate the risk of public health threats posed by aquaculture products, it is essential to gain a deeper understanding of the occurrence and dynamics of human pathogenic Vibrio. As part of a monthly qualitative Vibrio spp. monitoring programme in a German biofloc aquaculture in 2022, the occurrence and diversity of *V. parahaemolyticus* in shrimps and water samples over a period of one year were determined by conventional typing, macrorestriction profiling (PFGE) and whole-genome sequence (WGS) analysis. An additional investigation was conducted 16 months after the last monitoring sampling in order to provide a comparison. The analysis of the *V. parahaemolyticus* isolates revealed minimal variability in their SfiI-PFGE profiles across all sampling approaches, indicating the persistence of a predominant clonal lineage well adapted to the conditions of the established biofloc. In-depth characterisation of a selection of representative isolates was conducted by plasmid determination and WGS, confirming that the observed differences were mainly attributed to the presence of acquired plasmids, which were identified in some of the isolates. The presentation will provide insight into the micro- and macroevolution events of *V. parahaemolyticus* in the German biofloc aquaculture, and the impact of other Vibrio species on foodborne infection found during the monitoring will be briefly addressed.

2.26 *Arcobacter cryaerophilus* exhibits pathogenic potential in human colonic cell lines

Antonia Bachus[1], Sarah Beyer[1], Roland Bücker[2], Soroush Sharbati[3], Thomas Alter[1], Greta Gölz[1]

[1] Insitute of Food Safety and Food Hygiene, Freie Universität Berlin, Berlin, Germany
[2] Clinical Physiology/Nutritional Medicine, Charité – Universitätsmedizin Berlin, Berlin, Germany
[3] Insitute of Veterinary Biochemistry, Freie Universtität Berlin, Berlin, Germany

The Gram-negative bacterium *Arcobacter cryaerophilus* has been isolated from a variety of sources, including different types of food. In recent years, case reports of intestinal and extraintestinal infections have led to its recognition as an emerging foodborne pathogen. However, the pathogenic mechanisms leading to persistent watery diarrhea are still poorly understood. We therefore tested twelve A. cryaerophilus strains of different origins for their pathogenic potential in two human colon cell lines, HT-29/B6 and T84.

Investigation on the potential cytotoxicity of A. cryaerophilus revealed that approximately 20% of the strains tested were able to reduce the metabolic activity of both cell lines. Moreover, all tested strains successfully adhered to and invaded HT-29/B6 and T84 cells, showing strain-dependent index variations of up to two orders of magnitude.

To investigate the effect of A. cryaerophilus on the epithelial barrier function, transepithelial electrical resistance (TER) was measured. The results indicated a strain-dependent decrease in the residual TER, suggesting that an infection with certain strains contributes to the impairment of the epithelial barrier of the tested cell line. These findings were confirmed by immunofluorescence staining of the tight junction domain, revealing a reduced immunofluorescence signal of claudin-5 in infected cells.

In conclusion, we observed different pathogenic mechanisms of the studied A. cryaerophilus strains using different in vitro assays in two human colonic cell lines. The strain-dependent effects observed may explain key symptoms such as leak-flux diarrhea, and indicate the potential risk of A. cryaerophilus to human health.

2.27 An outbreak of foodborne botulism cause by commercially canned mushrooms from Russia

Martin B Dorner[1], Christina Frank[2], Sylvia Worbs[1], Martin Skiba[1], Laura Wilk[1], Maximilian Steinberg[1], Hendrik Wilking[2], Brigitte G Dorner[1]

[1] German National Consultant Laboratory for Neurotoxin-producing Clostridia (Botulism, Tetanus), Robert Koch Institute, Berlin, Germany
[2] Infectious Disease Epidemiology, Robert Koch Institute, Berlin, Germany

Foodborne botulism outbreaks induced by commercial foods are rare within the EU. We report on an outbreak among two persons in Germany from unrelated households in Mai/June 2024. Both persons were of Eastern European ethnicity and had been severely affected requiring intensive care with ventilation.

In both cases type A foodborne botulism could be confirmed based on the presence of an BoNT/A2-producing C. botulinum strain from stool and BoNT/A from serum. In collaboration with the local health authorities, food items and leftovers were collected from the households and supermarkets. Glass jars of canned white mushrooms (Russula delica) locally produced in the Altai Krai (Russia) were identified as the source of the outbreak based on the presence BoNT/A2-producing C. botulinum strains and BoNT/A in a number of jars. Russia has been informed via IHR channels. Interestingly, a search across Russian (social) media revealed that a similar product (Russula delica from the Altai) of the same producer had been recalled in Russia earlier the year after a botulism outbreak in Irkutsk.

A comparison of the BoNT/A2 amino acid sequence with other A2 sequences (NCBI databases) revealed that the sequence contained at least one substitution compared to all other A2 sequences. Noteworthy, a single identical A2 sequence deposited by a Russian institution was found.

In summary, mushrooms can be naturally contaminated with spores of BoNT-producing clostridia and canned mushrooms have been incriminated in botulism outbreaks in the past. Nowadays, commercial products are usually not involved in foodborne botulism outbreaks within the EU due to the high safety standards applied. However, foreign (non-EU) products from countries with less stringent safety or surveillance systems may enter the market, particular in niche areas. With respect to the current security situation an efficient communication and early product warning between the EU and non-EU countries might be challenged.

3 Abstracts – poster presentations

3.1 Monitoring of ESBL/AmpC-*E. coli* in the pork meat production chain in Germany: Impact of antimicrobial use

Carolina Plaza-Rodriguez[1], Alexandra Irrgang[1], Matthias Flor[1], Tasja Crease[1], Mirjam Grobbel[1], Ulrike Binsker[1], Annemarie Kaesbohrer[1], Katja Alt[1,2], Bernd-Alois Tenhagen[1]

[1] German Federal Institute for Risk Assessment (BfR), Department 4: Biological Safety, Unit 43: Epidemiology, Zoonoses and Antimicrobial Resistance
[2] German Federal Ministry of Food and Agriculture (BMEL), Department 3: Food Safety and Animal Health, Division 314: Meat and food hygiene

The presence of ESBL/AmpC-*E. coli* in livestock has raised concerns about its impact on public health. The objective of this study was to provide an overview on the presence of these bacteria in the pork meat production chain in Germany and to explore the impact of antimicrobial use (AMU) on the occurrence of multidrug resistant bacteria in fattening pigs.

Within the framework of the National Zoonoses Monitoring, caecal samples from fattening pigs at slaughter and pork meat samples at retail were taken in odd-years (2015-2023). Selective isolation of ESBL/AmpC-*E. coli* was carried out using EU wide harmonized procedures, followed by broth microdilution (2015-2019) and whole genome sequencing (2021-2023). Treatment frequency (average annual days of antimicrobial use) with aminopenicillins and 3rd/4th generation cephalosporins was used to evaluate the association between AMU and the occurrence of ESBL/AmpC-*E. coli* in the studied animal population.

ESBL/AmpC-*E. coli* was found in 46.2% of the caecal samples at slaughter and in 5% of the meat samples taken at retail, indicating limited cross-contamination during slaughter. Since 2015, no significant differences in the prevalence of ESBL/AmpC-*E. coli* has been observed in both sample types, despite a significant reduction in treatment frequency with aminopenicillins and 3rd/4th generation cephalosporins in pigs since 2014 (amoxicillin by 59.3%, ampicillin by 99.3%, cefquinome by 94.5% and ceftiofur by 93.1%).

Our findings highlight that while the pig slaughter process limits cross-contamination, it does not eliminate the transfer of ESBL/AmpC-*E. coli* from the caecal content to the meat. Additionally, the persistent prevalence of these bacteria in fattening pigs, despite reduced AMU suggests that factors beyond the consumpiton of aminopenicillins and cephalosporins may be of major relevance for the presence of these bacteria in the pork production chain.

3.2 Applications of bacteriophages as biocontrol agents against spoilage-causing Pseudomonas

Chiara Krühne, Stefanie Gieschler-Lübbehüsen, Frank Hille, Erik Brinks, Charles M. A. P. Franz

Max Rubner-Institut - Department of Microbiology and Biotechnology

Despite strict hygienic regulations, heat-stable enzymes produced by spoilage-causing Pseudomonas species remain a problem in the milk production industry. Milk components such as fats and proteins are broken down by the enzymes, which leads to adverse quality changes and a reduced shelf life of dairy products. A particular challenge in combating pseudomonads is their ability to form biofilms. Bacteriophages – viruses that specifically infect and lyse bacteria – represent a promising option for combating pseudomonads. In our work, we classify and characterize 16 newly isolated Pseudomonas phages. We tested individual host spectra and the ability of the phages to lyse or degrade planktonic bacteria and mono-species biofilms. The results show a large taxonomic and morphological diversity of phages, with the majority being head-tail phages belonging to the class Caudoviricetes. The host spectrum analysis showed that jumbo phages with a genome of over 200,000 bp exhibited the largest host spectra, whereas smaller phages infected only a few bacterial strains. Initial biofilm experiments with single phages and phage cocktails did not yield a significant reduction of host cells, but showed that the phages were still able to penetrate biofilms and infect embedded host cells. In contrast, a preventive phage application on yet unestablished biofilms revealed a strong inhibition of further biofilm formation. Additional experiments will provide insight into the optimal application strategy required for efficient phage treatment and the minimization of biofilm growth of a variety of spoilage-relevant pseudomonads.

3.3 Characterization of the pathogenic potential of *Campylobacter coli* clades

Sarah Beyer[1], Soroush Sharbati[2], Thomas Alter[1], Greta Gölz[1]

[1] Institute of Food Safety and Hygiene, Freie Universität Berlin
[2] Institute of Veterinary Biochemistry, Freie Universität Berlin

Campylobacter infections in humans are a major cause of foodborne gastroenteritis, of which approximately 11% are due to *Campylobacter coli* (*C. coli*) infections. *C. coli* can be categorized into different clades and clade 1A strains have been identified in human cases of campylobacteriosis. In contrast clade 2 and 3 strains were less frequently detected in human cases but are highly prevalent in the environment. The aim of this study is to investigate whether the pathogenic potential of *C. coli* clades 2 and 3 is lower than that of clade 1A. A WST-1-assay for the cytotoxic potential as well as adhesion- and invasion-assays were performed in vitro using two different human colon cell lines (HT-29/B6, T84) along with *C. coli* isolates belonging to clades 1A, 1C, 2 and 3, respectively. All C. coli isolates were able to adhere to and invade both cell lines, with isolate-dependent variances. The cytotoxic potential of *C. coli* clade 3 isolates was more pronounced compared to isolates from the other clades, as they strongly reduced the metabolic activity of the HT-29/B6 cells as early as 18 h after infection. In contrast, a comparable reduction induced by most strains from the other clades was only observed after 48 h. This is also supported by the results with the T84 cell line, where only the clade 3 isolates induced a high reduction of the relative metabolic activity after 48 h. In conclusion, our results indicate a higher cytotoxic potential for clade 3 isolates, whereas no obvious difference in the adhesion or invasion potential could be detected compared to the other clades. Therefore, the lower detection rate of clade 3 isolates in human campylobacteriosis cases seems to be related to factors other than those investigated in our study.

3.4 Changes in gene expression of *Escherichia coli* ATCC 8739 in strawberry nectar after thermal and non-thermal treatments

Astrid Gędas, Agnes Weiss

Food Microbiology, Hamburg School of Food Science, University of Hamburg, Ohnhorststrasse 18, 22609 Hamburg, Germany

Strawberries are a rich source of bioactive compounds, such as vitamin C and phytochemicals. However, traditional thermal treatments used to preserve fruit juice and nectar also lead to significant nutrient losses. Therefore, alternative non-thermal methods are being explored to inactivate foodborne pathogens, like *Escherichia coli*, while preserving the nutritional value. Despite their potential, the effects of these technologies on bacterial cells remain insufficiently understood. In this study, changes in global gene expression of *E. coli* ATCC 8739 inoculated in strawberry nectar were analyzed following thermal and non-thermal treatments. Three preservation methods, each with two sets of parameters, were selected: thermal treatment, high-pressure processing (HPP), and moderate-intensity pulsed electric field (PEF).

The highest microbial inactivation was achieved with HPP at 400 MPa for 1 min, reducing bacterial counts by 5.0±0.3 log cfu/mL, followed by thermal treatment at 60°C for 200 s, with a 4.4±0.2 log cfu/mL reduction, while no inactivation was observed with PEF at 6 kV/cm. Gene expression analysis revealed that thermal and HPP treatments induced similar molecular responses in the tested strain. After both methods, the most highly overexpressed genes encoded outer membrane proteins, potentially triggering the envelope stress response. Although no microbial inactivation was observed after PEF treatment, strong transcriptomic responses were detected, particularly in genes associated with membrane integrity and metabolic activity. Several overexpressed genes related to ABC transporters, outer membrane proteins, and lipoproteins were identified, which may enhance the strain's virulence.

The findings of this study give insights into the stress response mechanisms induced by thermal and non-thermal treatments. However, further research is needed to assess the long-term effects on bacterial populations.

3.5 Compatibility of WGS data from Illumina and Ion Torrent technology in genome comparison analysis of *Listeria monocytogenes*

Stefanie Lüth, Carlus Deneke

Department of Biological Safety, German Federal Institute for Risk Assessment

Whole genome sequencing (WGS) has become the key approach for molecular surveillance of *Listeria monocytogenes* (Lm). Genome comparison analysis can reveal transmission routes that cannot be found with classic epidemiology.

Widespread standard for use in genome comparison analysis is data from short read sequencing, generated on Illumina or Ion Torrent devices. To date, little is known on the compatibility of data from both platforms. This knowledge is essential when it comes to the central analysis of data, e.g. in the case of outbreaks.

We used WGS data from 47 Lm isolates of the strain collection of the German National Reference Laboratory for Lm, generated on either Illumina or Ion Torrent devices, to analyse the impact of the sequencing technology on downstream analyses. In our study, only the assembler SPAdes delivered qualitatively comparable results. In the gene-based core genome multi locus sequence typing (cgMLST), the same-strain allele discrepancy between the platforms was 14.5 alleles on average, which is well above the threshold of 7 alleles routinely used for cluster detection in Lm. Application of a strict frameshift filter could push the mean discrepancy below this threshold, but reduced discriminatory power. The impact of the platform on the read-based single nucleotide polymorphism (SNP) analysis was lower than on the cgMLST. Overall, it was possible to improve compatibility in various ways, but perfect compatibility could not be achieved.

3.6 Fresh bioprotection of ready to eat products

Juliane Kretzschmar, Laura Teichmann, Tobias Neddermann

Competence Center Cultures, NovaTaste Production GmbH

Industry Abstract

In 2023, the European Food Safety Authority recorded an alarming peak in Listeria infections since 2007. In response to these developments, the EU Commission has tightened the food safety criteria for ready to eat foods that support the growth of *Listeria monocytogenes* with Regulation EU 2024/2895. Thus, food cultures with a protective function become even more important, as their metabolic functions are not only crucial for the maturation, flavour, colour and texture of fermented foods, but can also give them a competitive advantage over food-spoiling organisms. Furthermore they are able to inhibit certain pathogenic organisms such as *L. monocytogenes* through the production of antimicrobial peptides.

Aim of the study is to improve food safety, reduce food waste and ensure consumer health by establishing protection against *L. monocytogenes* in ready to eat products. Challenge tests were carried out based on the technical recommendation of the European Union Reference Laboratory for *L. monocytogenes*. The effects of food culture BITEC® B fresh aquatic in smoked salmon and food culture BITEC® B Safe Knack in hotdogs were examined in these studies. A late entry of Listeria in the production process, for example due to recontamination when products are sliced or packed was analyzed. In both challenge tests, it was clearly shown that the used lactic acid bacteria inhibit growth of *L. monocytogenes*. Additionally they also improved the sensory properties of the final product, even when stored for a longer period.

The importance of additional measures to increase food safety for risky ready to eat products such as smoked salmon or hotdogs is undisputed. As previously described, the use of food culture inhibits Listeria growth until the expiry date and beyond. As a result, the food can be classified according to EU regulation 2073/2005 as a food unable to support the growth of *L. monocytogenes*.

3.7 ZooNotify – An interactive data tool for searching and visualizing zoonoses monitoring results along the food chain in Germany

Tasja Crease[1], Carolina Plaza-Rodriguez[1], Mahtab Iltarabian[1], Dominic Tölle[1], Katja Alt[2], Bernd-Alois Tenhagen[1]

[1] German Federal Institute for Risk Assessment (BfR), Department 4: Biological Safety, Unit 43: Epidemiology, Zoonoses and Antimicrobial Resistance
[2] German Federal Ministry of Food and Agriculture (BMEL), Department 3: Food Safety and Animal Health, Division 314: Meat and food hygiene

Data on the occurrence of zoonotic agents and related antibiotic resistance in the food chain in Germany are mainly available in Portable Document Format (PDF) reports. This limits their accessibility and reusability in science and for risk assessment. ZooNotify makes existing zoonoses data findable, accessible, interoperable and reusable (FAIR).

ZooNotify comprises data from the zoonoses monitoring along the food chain and the Salmonella control programmes in poultry flocks. It provides information on prevalence, antibiotic resistance and typing of zoonotic agents based on samples taken at various stages of the food chain, such as primary production, slaughterhouses and retail. The majority of the data is related to livestock and foods of animal origin. However, data is also available on feed, wild animals and plant-based foods.

Currently, ZooNotify contains antibiotic resistance and typing data for 34.956 and 16.619 bacterial isolates from animals and food, respectively. Additionally, it provides more than 600 prevalence estimates for different zoonotic agents along the food chain. The data is partially aggregated, but we are currently making efforts to provide more unaggregated data.

ZooNotify allows customized data searches based on user interests, with selected data available for download. The tool also offers intuitive data visualisations, including time trends, with the option to download generated figures. Each section of ZooNotify is accompanied by clear and concise explanations, ensuring ease of use and comprehensive understanding of the data. Users are encouraged to reuse the data and generated figures provided by ZooNotify.

The continuous development of ZooNotify aligns with the EU strategy for open data and forms an important basis for improved risk assessment and the protection of animal and consumer health.

3.8 Cronobacter in non-PIF beverages – data and review of the literature

Svea Ose[1], Alexander Bartel[3], André Goehler[2], Eva Spindler-Raffel[1], Matthias Fischer[4]

[1] Institute for Food Safety, Hochschule Geisenheim University
[2] National Reference Laboratory for *Escherichia coli* including verotoxin producing *E. coli*, German Federal Institute for Risk Assessment
[3] Institute for Veterinary Epidemiology and Biostatistics, Freie Universität Berlin
[4] Food Microbiology, Host-Pathogen-Interaction, German Federal Institute for Risk Assessment

Cronobacter spp can cause rare but severe infection such as meningitis, septicaemia and necrotising enterocolitis in neonates and very young children. The most frequently reported infection source is powdered infant formula (PIF). Whereas infection dose for Cronobacter infections of 100 to 10000 cells were suggested. Other sources of infection are utensils or formula preparation like bottles or tits, water and handling failures. Aside from PIF, water and herbal infusions such as in fennel and chamomile were offered to infants and young children. However limited knowledge exists for herbal tea and tea beverages as well as water.

We further aimed to analyse the growth characteristics of different Cronobacter (N=12) species in fennel and chamomile herbal infusions. These beverages were prepared at brewing temperatures (100°C and a brewing time of 5 min) and inoculated with low levels of Cronobater spp. The growth parameters of the bacteria at room temperature were measured, calculated and compared. The used strains differed in their ability to grown in the tea infusions, whereas Chamomile infusions were able to negatively affect the growth of different Cronobacter species. Overall, the lag times ranged from ~8 to 12 hours.

As a consequence, the storage of herbal infusions for infants at room temperature is critical regarding the possible bacterial growth. Therefore, the storage time should be strictly limited. An overnight storage is definitely too long as the growth starts after about 8 hours.

3.9 Two decades of Salmonella surveillance in poultry food chain of Marche Region: Antimicrobial resistance and virulence profiles

J. Riaz[1], M. Napoleoni[2], G. Brandi[1], G.F. Schiavano[3], G. Amagliani[1]

[1] Department of Biomolecular Sciences, University of Urbino Carlo Bo, Urbino
[2] Regional Reference Center for Enteric Pathogens Marche, Istituto Zooprofilattico Sperimentale dell'Umbria e delle Marche "Togo Rosati", Perugia
[3] Department of Humanities, University of Urbino Carlo Bo, Urbino

Background

EU surveillance data indicate that Salmonellosis is the second most frequently reported gastrointestinal infection. This study aims to provide insights into the evolution and trends of resistance patterns and virulence features in Salmonella spp. from the poultry chain in the Marche region, Italy, covering eighteen years (2005-22).

Methodology

A total of 400 Salmonella strains for routine surveillance (2005-2022) were collected from veterinary samples and food, human clinical samples, and surface water. AMR profiles were obtained through the disk diffusion method (CLSI, 2022). PCR-based amplification of twelve virulence gene targets was performed that included the genes of the Salmonella Pathogenicity Islands SPI-1 (invA, sopB, sopD, sipA, sipB, sipD), SPI-2 (ssaR, sifA, spvB), outer-membrane protein (tolC) and flagellar proteins (flgK, flgL).

Results

A rising trend of AMR was observed against all antimicrobial classes (n. 7) in human strains, and five classes in Food-Vet strains. MDR data revealed that 72 % of vet-food, 74 % of humans, and 64 % of surface water strains were resistant to at least one antimicrobial agent, however, 56%, 62%, and 55% of these isolates were multidrug-resistant (MDR). Different patterns of virulence gene distribution (36=human, 50=surface water, 79=Food-Vet) have been observed. A higher frequency of tolC, sipA, invA, and sipB were found in surface water strains and Food-Vet whereas, spvB, sipD and sopD were the least prevalent genes. sopB, sipA, sipB, and tolC were always (100%) present in all human strains. The virulence genes were highly prevalent in S. Typhimurium in surface water, S. Enteritidis in human samples, and S. Infantis in the food-Vet strains.

Conclusions

Diverse virulence gene patterns regardless of serovars and years of isolation reflect the evolutionary dynamics of Salmonella. Rising AMR trends in human and Food-Vet origin strains increase their pathogenic potential, posing a significant threat to public health.

3.10 Using Whole Genome Sequencing to monitor *Listeria monocytogenes* along the food chain and to identify causative foods in listeriosis outbreaks

Sylvia Kleta[1], Stefanie Lüth[1], Marlen Adler[1], Vanessa Wollny[1], Carlus Deneke[2], Maria Borowiak[2], Holger Brendebach[2], Burkhard Malorny[2], Szilvia Neuhaus[1]

[1] German Federal Institute for Risk Assessment, Department Biological Safety, Unit Product Hygiene and Disinfection Strategies, National Reference Laboratory for Listeria monocytogenes
[2] German Federal Institute for Risk Assessment, Department Biological Safety, National Study Centre for Sequencing in Risk Assessment

Whole Genome Sequencing (WGS) is a powerful tool for comprehensive genetic characterization of bacterial pathogens. Downstream analyses such as core-genome multi-locus sequence typing provide high resolution for assessing genetic relatedness between isolates, which is particularly useful in strain comparison along the food chain and during outbreak investigations. WGS is especially advantageous for tracking *Listeria monocytogenes* (Lm), a widespread pathogen found in various foods due to its regular introduction during food production processes.

The National Reference Laboratory (NRL) for *Listeria monocytogenes* at BfR is routinely sequencing isolates from official controls and sometimes from manufacturers' own controls. Open-source, in-house pipelines at BfR allow mostly automated high-throughput analyses. To date, around 7,500 Lm isolates from food and food production environments have been sequenced at BfR. These results are essential for monitoring Lm throughout the food chain in Germany.

In food production environments, Lm often persists in hard-to-clean areas, leading to cross-contamination during processing. WGS-based strain comparisons help to identify such niches and to trace the spread of the pathogen which is crucial for eliminating Lm from manufacturing facilities and food products.

By comparing non-clinical isolates from food, animals and the environment with clinical isolates from human listeriosis cases, infection chains can be traced. In close collaboration with the food sector and public health partners, the NRL has successfully identified the potential source of numerous listeriosis outbreaks in Germany. Matching isolates came mainly from meat and fish products. WGS often provided the first clue about the causative foodstuff. This work underscores the importance of metadata integration and cross-sector data sharing for accurate interpretation of WGS comparisons and for understanding the infectious disease epidemiology of listeriosis in Germany.

3.11 Pathogens in foods database: web resources for assessing the occurrence of microbiological hazards in foods surveyed in European countries

Pauline Kooh[1], Ana Sofia Faria[2], Anne Thebault[1], Laurent Guillier[1], Winy Messens[3], Vasco Cadavez[2], Ursula Gonzales-Barron[2]

[1] Risk Assessment Department, French Agency for Food, Environmental and Occupational Health & Safety (ANSES), 14 rue Pierre et Marie Curie, Maisons-Alfort, 94701 Paris, France
[2] CIMO, LA SusTEC, Instituto Politécnico de Bragança, Campus de Santa Apolónia, 5300- 253 Bragança, Portugal
[3] European Food safety Authority (EFSA), 1A Via Carlo Magno, 43126 Parma, Italy

Studies on the pathogens occurrence in the farm-to-fork chain are essential for the development of risk assessment models, risk management tools and meta-analysis by food researchers and food safety authorities. However, the existing data are mostly dispersed, non-standardized or not easily accessible.

Pathogens-in-Foods (PIF) is a dynamic database of occurrence data (prevalence and enumeration) of pathogens in foods randomly sampled across Europe. Data are periodically extracted from peer-reviewed articles retrieved through systematic literature searches using a publicly available protocol describing the search and screening process. Eligible primary studies are quality assessed and verified data are extracted into the database, according to a predefined categorization protocol that includes general study characteristics, food categorization (harmonized with EFSA's FoodEx2 system), pathogen information, microbiological methods, and outcomes (prevalence and/or enumeration results).

The PIF database covers 26 hazards including bacteria (Salmonella, *Campylobacter,* Shiga-toxin producing *Escherichia coli, Listeria monocytogenes, Yersinia enterocolitica, Bacillus cereus, Clostridium perfringens* and *Staphylococcus aureus*), viruses (Norovirus, Hepatitis A and E viruses), protozoans (Cryptosporidium, Giardia and Toxoplasma gondii), and nematodes and other parasites in fishery products (Anisakis, Contracaecum, Phocanema, Pseudoterranova, Opisthorchis, Metorchis, Pseudamphistomum, Dibothriocephalus, Bolbosoma, Corynosoma, Kudoa and Cryptocotyle). In 2025, PIF database will be populated with data on the occurrence of Vibrio parahaemolyticus, Vibrio vulnificus and non-O1, non-O139 Vibrio cholerae in seafood. Accessible through a web application (https://pif.esa.ipb.pt/), PIF enables data retrieval according to several relevant variables, visualization and statistics analysis through interactive dashboards.

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3.12 Viruses in food: strategies for detection and inactivation studies

Hui Zhi Low, Christina Böhnlein, Charles M. A. P. Franz

Department of Microbiology and Biotechnology, Max Rubner-Institut, Federal Research Institute of Nutrition and Food, Hermann-Weigmann-Str. 1, 24103 Kiel, Germany

Since the description of the “Winter Vomiting Disease” in 1968, human noroviruses are still refractory to study due to a lack of virus cultivation methods, making it difficult to investigate aspects such as the virus life cycle or inactivation conditions. Nevertheless, noroviruses are important foodborne viruses that are the leading cause of gastroenteritis worldwide, with WHO estimates of 685 million cases annually. A food virus laboratory at the Max Rubner-Institute was recently founded to contribute to the advancement of virus detection methodologies, as well as to study virus inactivation conditions in food, especially that of norovirus.

When establishing the detection method of food viruses according to ISO 15216-2:2019, we determined that expensive one step real time quantitative PCR reagents (rt-qPCR) represent a financial bottleneck in virus detection, owing to the necessity of a plethora of controls in order to exclude false negative results. We therefore created our own rt-qPCR mix based on commercially available enzymes, which was 3x more cost-effective and performed better than the RNA Ultrasense kit, which is proposed in the ISO protocol. Our in-house rt-qPCR mix is also adaptable to multiplex detection. Moreover, we are developing a novel norovirus culture system with the aim of studying inactivation conditions in food matrices. Human intestinal enteroids have emerged as a promising way to analyze norovirus growth and inactivation, however they are very costly and laborious to establish, requiring the use of human biopsy samples which most labs do not have access to. With the long-term goal of generating replication-incompetent but infectious norovirus particles, we started off with generating stable immortal mammalian cell lines expressing norovirus capsid proteins. We will present our first results and a sketch of future plans for norovirus generation and inactivation studies.

3.13 Validierung Cluster-spezifischer PCR-Screening-Assays für *E. coli* in Hackfleisch

Tim Neumann, Elisabeth Schuh, Michaela Projahn

Nationales Referenzlabor für *Escherichia coli* einschließlich VTEC, Abteilung für Biologische Sicherheit, Bundesinstitut für Risikobewertung, Berlin

Lebensmittelausbrüche verursacht durch Shigatoxin-produzierende *E. coli* erfordern eine schnelle Identifikation der Infektionsquellen. Dafür müssen die verursachenden Stämme (Clusterstamm, CS) identifiziert werden. Diese Studie untersuchte PCR-basierter Assays zum Nachweis spezifischer CS, die ursprünglich für Reinkulturen etabliert, und deren Anwendung auf Mischkulturen, am Beispiel von Hackfleisch, getestet wurde.

Es wurde Hackfleisch mit bekannten CS und Nicht-CS in zwei Konzentrationen (102 und 104 CFU/ml) kontaminiert. Koch-DNA wurde nach Inkubation (t-0 h und t-24 h) in einem nicht-selektiven Flüssigmedium (PW1) sowie nach weiterer Anreicherung (t-48 h) auf Selektivplatten (TBX) gewonnen. Fünf Assay-PCRs wurden eingesetzt: drei konventionelle PCRs (eine Standard-PCR und zwei Melt-MAMA-Varianten) sowie zwei real-time PCRs (TaqMan, SYBR-Green). Zudem erfolgte eine MALDI-Speziesbestimmung der Hintergrundflora.

Die drei konventionellen PCRs zeigten CS- und nicht-CS spezifische Banden, jedoch traten unspezifische Banden und Fragmentierungen auf, wobei einige eine ähnliche Basenpaargröße zum CS aufwiesen. Die SYBR-Green Assays lieferten Schmelzkurven, die sich in den Schmelztemperaturen nicht eindeutig voneinander unterscheiden ließen. Der TaqMan-Assay hingegen zeigte ausschließlich eine spezifische Amplifikation der CS, ohne Kreuzreaktivität mit der Hintergrundflora.

Somit erwies sich der TaqMan-Assay als die zuverlässigste Methode zur spezifischen Detektion von CS in Mischkulturen aus Hackfleisch. Konventionelle Assays konnten CS und Nicht-CS anhand ihrer Bandengröße differenzieren, waren jedoch anfällig für Kreuzreaktionen. Die SYBR-Green Assays ermöglichten eine Differenzierung der CS, erforderten jedoch eine genaue Analyse zur Abgrenzung der Hintergrundflora. Die MALDI-Analyse zeigte das Vorhandensein von *E. coli* in der Hintergrundflora, was zusätzliche Banden in den konventionellen PCRs und die Kreuzreaktion der Primer erklären könnte.

3.14 Shigatoxin-producing *E. coli* in ready-to eat food – the unpleasant ingredient

Elisabeth Schuh, André Goehler, Sebastian Steffan, Sabine Haby, Dana Schmidt, Annica Seemann, Katja Drache, Michaela Projahn

German Federal Institute for Risk Assessment (BfR), Department Biological Safety, Unit Food Microbiology, Host-Pathogen-Interactions, National Reference Laboratory for *E. coli* including VTEC, Max-Dohrn Str. 8-10, 10589 Berlin, Germany

Shigatoxin-producing *E. coli* (STEC) are zoonotic pathogens with main reservoir in ruminants. STEC infections are mainly caused by ingestion of contaminated food, resulting in symptoms like (bloody) diarrhoea and may proceed to life threatening hemolytic uremic syndrome. Key virulence factor is the Shiga toxin (encoded by stx1 or stx2 and variants) and as additional aggravating factor adhesion protein intimin (encoded by eae).

Our focus were STEC isolates from ready-to-eat or (likely) raw consumed food sent routinely to the German national reference laboratory for *E. coli* between 2019 and 2023 by the official state laboratories. The aim was a pathogenicity assessment of STEC in this food category using routine typing data (e.g. stx genes, eae gene, serotype).

We analyzed 215 unique STEC isolates from 13 different federal states. The highest numbers were from raw sausages (N=64) partly with game meat (N= 14 thereof), followed by (raw milk) cheese (N=62), minced meat from cow (N= 36; e.g.“Tartar”) or pig (N= 11; e.g. “Mett”), fresh produce (N=21 e.g. lettuce) or others (N=20) like mixed salads, sandwiches/rolls, spices and one isolate from butter.

Predominant stx-types were stx2 (N=141) with mainly subtype stx2b, followed by stx1 (N=54) with mainly stx1a and a combination of stx1+stx2 (N= 20) mainly stx1c+stx2b. Additionally, 23 isolates were positive for the eae gene. Serogroups most prevalent in clinical isolates (O146, O91, O103, O157, O145) were detected in 33 isolates (none of O26).

Based on FAO/WHO experts´ STEC risk assessment, we categorized eight isolates within the highest pathogenicity rank equipped with at least stx2a and eae. Four isolates from cheese (two O148:H28, O150:H2, O177:H25), three from minced meat intended to be eaten raw (O157:H7, O177:H11, O115:H25) and one from ready-to eat salad (O157:H7). Regarding the relatively low infectious dose of STEC, contaminated ready-to eat food is of high risk for an STEC infection especially in vulnerable consumers.

3.15 Bacteriophage insensitive mutants of clinical and food-associated *Listeria monocytogenes* strains using a commercial phage product

Christoph Brieske, Erik Brinks, Christina Böhnlein, Hui Zhi Low, Charles M. A. P. Franz

Department of Microbiology and Biotechnology, Max Rubner-Institut, Federal Research Institute of Nutrition and Food, Hermann-Weigmann-Str. 1, 24103 Kiel, Germany

Background: In recent years, phage products have been used for biocontrol of Listeria (L.) monocytogenes to improve food safety.

Since little is known about resistance of *L. monocytogenes* to commercially available Listeria phage products, the aim of this study was to investigate the impact of such a phage product on clinical and food-associated *L. monocytogenes* strains recently isolated in Germany.

Methods: The emergence of bacteriophage-insensitive mutants (BIMs) to a P100 phage product was tested on soft agar at 20°C and 10°C. Colonies were isolated after several days and exposed to the phage to validate reduced phage-susceptibility by flow cytometric live/dead staining, spot assays and confocal microscopy. Growth curves of BIMs and wild-types (WTs) were generated by flow cytometric cell counting. In addition, BIMs and WTs were sequenced using whole genome sequencing. The antibiotic susceptibility of *L. monocytogenes* following the development of resistance to the phage product was also evaluated.

Results and Discussion: *L. monocytogenes* mutants with reduced phage sensitivity emerged after a few days both at 20°C and 10°C. Flow cytometric live/dead staining and spot assays confirmed a significantly lower bacterial reduction or no lysis of the BIMs after phage treatment compared to the respective WTs. Whole genome sequence analysis of 2 BIMs revealed mutations and deletions in a gene encoding a cell wall-associated protein. This Lmo1799 gene has recently been described in the literature and may contribute to the binding of phages to host receptors. This hypothesis is supported by reduced binding of phages to the BIMs using confocal microscopy. Three of 5 BIMs showed a slightly delayed growth, possibly due to fitness trade-offs. Two BIMs showed increased susceptibility to β-lactam antibiotics. Taken together, these results contribute to a better understanding of the development of phage resistance to a commercially available P100 phage product.

3.16 Microbiological criteria and food safety - a nationwide study of refrigerator temperatures in private households

Christina Böhnlein, Martina Hoffmann, Gregor Fiedler, Charles M. A. P. Franz, Jan Kabisch

Max Rubner-Institut, Department of Microbiology and Biotechnology, Hermann-Weigmann-Str. 1, 24103 Kiel; (2) German Federal Institute for Risk Assessment (BfR), Department Risk Communication, Max-Dohrn-Straße 8-10, 10589 Berlin

Commission Regulation (EC) No. 2073/2005 stipulates that food business operators can perform challenge tests, in particular for foodstuffs that may favor the growth of *Listeria monocytogenes*, in order to ensure compliance with food safety criteria throughout the entire shelf life of the food. The technical guideline of the EU Reference Laboratory for *Listeria monocytogenes* (EURL) describes the procedure for this in detail. It also states that a temperature of 10 °C should be used for storage tests at consumer level. Alternatively, national data on temperature regimes of domestic refrigerators can also be used. So far, however, no representative data have been available for Germany.

Therefore, MRI and BfR jointly conducted a nationwide study which included more than 1,000 private households in order to obtain reliable and statistically validated data on the temperatures encountered in domestic refrigerators in Germany. The participating households for the cohort-based survey were selected to be representative for the following criteria: region, household size, net monthly income and age of the person primarily responsible for food shopping and preparation. During the household visits, interviews were conducted and temperature data loggers were placed at a defined location in the fridge. The temperatures were recorded electronically for approximately 30 days and then subsequently analysed.

On average, the temperature in the middle of the refrigerator in German households was 6.6 °C, which corresponds to the recommended temperature range of 5 to 7 °C for that location. Temperatures between 3.5 °C and 10.5 °C were measured in 85% of the refrigerators tested. While a temperature of more than 10.5 °C was recorded in 5% of cases, the average refrigerator temperature in 10% of consumer households was less than 3.5 °C. However, differences between participant age groups were found in the recorded refrigerator temperatures. A lower mean refrigerator temperature (M = 6.3 °C) was measured in households of individuals aged 18 to 44 years when compared to those of individuals aged 45 to 64 years (M = 6.7 °C) or those of individuals aged 65 years and older (M = 6.9 °C). The correlation analysis also showed that a higher age of the person in charge of the household was associated with a higher observed average refrigerator temperature. In addition, 28% of persons aged 65 and above assumed that the optimal refrigerator temperature should be 8 °C or higher. This is a faulty assumption as this temperature is higher than the recommended maximum refrigerator temperature of 7 °C. In the other two age groups, only 19% and 22% of respondents were of the same opinion that 7.5 °C is the optimal refrigerator temperature. The findings of this study are based on representative data from consumer households, and thus for the first time provide reliable insights into actual temperature conditions of refrigerators in private households in Germany.

3.17 Generating bacteriophage insensitive mutants of *Listeria monocytogenes* on sausage-based products

Katharina Willenbücher[1], Taniesha A. Setiadi[2], Frank Hille[1], Ramona Nitzsche[2], Natalia Biere[1], Christian Hertel[2], Charles M. A. P. Franz[1]

[1] Max Rubner-Institut, Federal Research Institute of Nutrition and Food, Department of Microbiology and Biotechnology, Hermann-Weigmann-Straße 1, 24103 Kiel, Germany
[2] DIL Deutsches Institut für Lebensmitteltechnik e. V., German Institute of Food Technologies, Prof.-von-Klitzing-Str. 7, 49610 Quakenbrück, Germany

Introduction

Contamination of meat products with the pathogen *Listeria* (L.) *monocytogenes* is a rare occurrence, yet of significance due to the severity of Listeria infections and a high mortality rate, which averages approximately 7%. The reduction of Listeria during the production of meat products is imperative, and a combination of decontamination methods such as bacteriophage application and high-pressure pasteurization offers a viable approach. A salient concern for the use of bacteriophages is the emergence of bacteriophage-insensitive mutants (BIMs). These BIMs have the potential to be unresponsive to phage treatment, thus necessitating the development of novel mitigation strategies. This study presents the methodologies and preliminary outcomes of experiments designed to explore the emergence of BIMs in a sausage-based medium using two commercially available phage products.

Results

Treatment of Listeria at 7°C with phage products initially showed a complete reduction in growth. However, after prolonged incubation single colonies emerged, indicating phage resistance. The development of BIMs within the food compound, i.e. the sausage, is of interest as it can lead to recontamination of food and therefore poses a health risk. Hence, infection of Listeria strains by both phage products were conducted on sausage products, a sausage-based agar, a sausage liquid media and a meat agar. Colonies that survived infection were analysed for their sensitivity towards both phage products by comparing the infection efficiency of the colonies with the WT Listeria strain using optical density measurements with a Microplate reader.

Summary

So far, reduced phage sensitivity has not been confirmed for any of the isolated colonies post-infection, indicating that the bacteria survived by other means (e.g. insufficient exposure to the phage during the experiment) than resistance development. Further testing is necessary to analyse the emergence of BIMs on meat products.

3.18 Dose-response relationships in cereulide intoxications: Data from outbreak related and routine food testing

Hendrik Frentzel, Pascal Witt, Ylanna Kelner-Burgos, Marco Krämer

German Federal Institute for Risk Assessment

Introduction

Cereulide is an extremely heat stable toxin produced in food during growth of emetic *Bacillus cereus* group strains. Ingestion of cereulide leads to nausea, vomiting and occasionally to organ failures and even fatal outcome. The emetic dose is estimated to be 10 µg/kg body weight, whereas the acute reference dose is estimated to be 0.03 µg/kg body weight, corresponding to 1.8 ng/g of food. Reported cereulide concentrations in food samples associated with intoxications are mostly in the range of 1,000 ng/g. In our poster we present cereulide concentrations in food samples from outbreaks and routine testing together with colony counts and WGS based data of emetic isolates.

Material and Methods

Initial investigations and microbiological food testing were performed by the competent local authorities in Germany. Upon submission of food samples to the BfR, cereulide concentrations were determined by LC-MS/MS in accordance with ISO 18465 within the National Reference Laboratory for the Monitoring of Marine Biotoxins. Submitted B. cereus (s.l.) isolates were further characterized by PCR and WGS based methods within the Specialized laboratory for spore formers at the BfR.

Results

All outbreak associated samples contained rice as the main ingredient. Cereulide concentrations in outbreak samples equivalent to the consumed food ranged from 371 to 1,606 ng/g. Samples without association to illness contained ≤ 21 ng/g. Most cereulide containing samples showed B. cereus (s.l.) levels of ≥ 10,000 cfu/g. Emetic isolates comprised ST 26, 165 and 1065. The cgMLST derived allele distance between multiple isolates from the same incident was 0–4 (corresponding to 0–24 cgSNP), whereas unrelated isolates differed by a minimum of 16 alleles.

Acknowledgments

We gratefully acknowledge the submission of food samples, bacterial isolates and sample data to the BfR by the competent food control authorities in Germany.

3.19 Metabolic cross-feeding between naturally occurring bacteria in milk and *Brucella abortus* supports the proliferation of the highly contagious food-borne pathogen

Anne Stephan[1], Sabrina Gensberger-Reigl[2], Deborah Maus[3], Martin Blume[3], Sascha Al Dahouk[4], Monika Pischetsrieder[2], Dirk Hofreuter[1]

[1] German Federal Institute for Risk Assessment, Department of Biological Safety, Berlin, Germany
[2] Food Chemistry, Department of Chemistry and Pharmacy, Friedrich-Alexander-Universität Erlangen-Nürnberg (FAU), Erlangen, Germany
[3] Metabolism of Microbial Pathogens (P6), Robert Koch-Institute, Berlin, Germany
[4] Department 1 - Infectious Diseases, Robert Koch Institute, Berlin, Germany

Bovine brucellosis is a widespread zoonotic disease primarily transmitted by the consumption of unpasteurized dairy products from cow's milk contaminated with *Brucella abortus*. Although B. abortus is viable in milk for several days, the effect of milk on its metabolic activity and propagation is unknown.

We identified in B. abortus several proteases that may contribute to the degradation of milk proteins by whole genome sequencing. Moreover, we demonstrated that B. abortus proliferates in commercial cow's milk with an increase of several magnitudes over 72 hours. To examine how the proteolytic activity of *B. abortus* facilitates the utilization of milk as a growth substrate, changes in the peptide composition of commercial cow's milk upon cultivation with B. abortus were examined by LC-MS analysis.

Although casein proteins are major milk components, we observed that B. abortus was unable to grow efficiently in minimal medium supplemented with uncleaved casein as sole carbon and energy course. However, bacteria could proliferate in minimal medium supplemented with tryptic-digested casein or casein hydrolysate as sole energy sources.

Thus, we hypothesized that the proteolytic activities of intrinsic milk bacteria might produce the casein-derived products being utilized by Brucella during their growth in milk. We therefore cultivated bacteria from fresh, unpasteurized cow's milk and identified isolates by MALDI-TOF MS and NGS analyses. Among the bacterial species found was *Staphylococcus sciuri*, which exhibited pronounced proteolytic activity upon cultivation on milk agar plates. We incubated the isolated milk bacteria in casein-containing minimal medium to produce spent media. *B. abortus* growth was notably enhanced by the spent medium derived from the proteolytically active *S. sciuri*. This observation suggests that *B. abortus* takes advantage of the casein-derived amino acids and peptides generated through the catabolic activity of *S. sciuri* in cow's milk.

3.20 The effect of community diversity on bacterial spatial patterns in the phyllosphere

Johannes Fuchs, Rudolf Schlechter, Mitja Remus-Emsermann

Institute of Microbiology and Dahlem Centre of Plant Sciences, Department of Biology, Chemistry, Pharmacy, Freie Universität Berlin, Berlin, Germany

Bacterial communities colonizing leaf surfaces, the phyllosphere, affect host health by enhancing nutrient acquisition, stress tolerance and pathogen proliferation. However, there is a general lack of understanding about the underlying processes of bacterial community assembly in the phyllosphere. Building upon previous research from our group, my work aims to expand the current knowledge of the ecology of bacterial community diversity in the phyllosphere and its influence on bacterial community spatial structures. I will inoculate the model plant Arabidopsis thaliana Col-0 with synthetic communities consisting of a core community of eight bacterial strains that will be supplemented by additional bacteria mimicking environmental bacterial communities. Two core community strains are genetically modified to constitutively express fluorescent proteins. Using fluorescence microscopy combined with image cytometry and spatial statistics, I will determine the effect of the different synthetic communities on the labelled bacteria strains. This will allow me to understand the relation between spatial distribution and bacterial community complexity. I will perform my measurements in a time-dependent fashion to also unravel the effect of community development on spatial arrangements. My research will provide deeper insights into bacterial interactions and community dynamics at the single cell resolution under nature-like conditions, improving our understanding and predictability of phyllosphere microbial ecology.

3.21 Towards a new sequencing era - Oxford Nanopore Technologies Sequencing as a method for bacterial characterisation and outbreak investigation?!

Maria Borowiak, Jennie Fischer, Alexandra Irrgang, Stefanie Lüth, Michaela Projahn, Kerstin Stingl, Burkhard Malorny, Carlus Deneke

German Federal Institute for Risk Assessment, Department Biological Safety

Introduction

Due to the low acquisition and sequencing costs, Oxford Nanopore Technologies (ONT) sequencing is attracting increasing interest from microbiology laboratories. In recent years, however, high error rates have hampered the routine use of ONT in bacterial characterisation and outbreak investigation. Recently, ONT announced to have overcome this obstacle with the latest developments in basecaller software and models. To investigate this, a test set of two Salmonella, Listeria, *E. coli* and Campylobacter isolates each was sequenced using ONT and Illumina techniques to compare the two data sets. In a follow-up study, a larger number of isolates is currently in the process of being sequenced and compared.

Materials and Methods

ONT libraries were prepared in triplicates using the SQK-RBK114.96 kit and sequenced on a P2 Solo device using a FLO-PRO114M flow cell. Subsequently, the data was basecalled using dorado v0.8.3 (model r1041_e82_400bps_sup_v5.0.0) and analysed using the MiLongA v1.4.0 pipeline. MiLongA performs read quality assessment, read filtering and assembly using flye v2.9.5. The correctness of the assemblies was further examined in comparison to the Illumina gold standard assemblies using cgMLST analysis.

Results

The results for the test data set consisting of eight isolates revealed that the accuracy of ONT assemblies is almost comparable to Illumina derived assemblies. In summary, 0 to 5 allele differences were detected between the respective ONT and Illumina assemblies. Results from the follow-up study with the large number of isolates are currently under investigation.

Conclusion

First results for the test data set look promising. However, this needs to be confirmed in our follow-up study, in which a larger data set is examined, as a higher ONT error rate may occur in individual, highly methylated isolates. This could lead to an incorrect conclusion – especially when ONT is applied in outbreak investigation.

3.22 Artificial Leaves: A Novel System for Studying Microbial Interactions on Leaf Surfaces

Evan Kear[1], Michał Bernach[1, 2], Eugenia Bosler[3], Christian Vehmann[3], Mitja Remus-Emsermann[1]

[1] Institut für Pflanzenphysiologie und Mikrobiologie, Freie Universität Berlin
[2] School of Biological Science, University of Canterbury
[3] Institut für Werkzeugmaschinen und Fabrikbetrieb, Technische Universität Berlin

Current research techniques in plant-microbe interactions are often limited by the complexity and heterogeneity of natural leaf surfaces. While these surfaces provide crucial environments for microbial colonization, their variability in nutrient composition, chemical properties, and physical structure makes controlled studies difficult. This limitation hinders the ability to draw general conclusions about bacterial aggregation, biofilm formation, and survival strategies on pant leaf surfaces.

To address these challenges, we are developing an artificial leaf surface that replicates the physical microtopography of plant leaves while eliminating confounding biological factors. These biomimetic surfaces provide a controlled system for investigating bacterial colonization patterns, enabling the isolation of topographical influences from other environmental variables.

This approach has significant implications for food safety and agricultural microbiology. Understanding how bacterial communities establish and persist on plant surfaces is crucial for managing microbial contamination in food production. Pathogenic bacteria, such as those responsible for foodborne outbreaks, exploit leaf surface structures to adhere and proliferate. By assessing bacterial interactions with different surface topographies, we can identify plant traits that either promote beneficial microbial communities or inhibit pathogen establishment. These insights may inform breeding programs for safer, more resilient crops and improve current sanitation strategies.

The ability to fabricate and modify leaf microstructures allows for tailored studies on microbial ecology, antimicrobial resistance development, and the role of surface features in microbial survival. By refining our understanding of bacterial behavior on plant surfaces, this work provides a foundation for innovative biocontrol approaches that reduce reliance on chemical interventions while enhancing food security.

3.23 Isolation and characterization of lactic acid bacteria for pea protein fermentation

Jana Hollmann, Herbert Schmidt

University of Hohenheim, Institute of Food Science and Biotechnology, Stuttgart, Germany

The increasing demand for plant-based protein alternatives highlights the need for optimized fermentation processes to improve the sensory and functional properties of pea protein. In this study, lactic acid bacteria were isolated from frozen peas and pea flours to identify substrate-adapted starter cultures for pea protein fermentation.

The isolates were taxonomically characterized by Random Amplified Polymorphic DNA PCR and 16S rRNA analysis. A rapid acidification test was performed in 24-well plates using 5 % (w/v) pea protein suspension with 1 % (w/v) glucose, where pH reduction was assessed using the indicator dye methyl red. To assess exopolysaccharide (EPS) production, the isolates were streaked on MRS agar containing glucose and sucrose and examined for ropy and mucoid non-ropy phenotypes using a loop test. Antimicrobial activity against common foodborne pathogens, including *Bacillus cereus* ATCC 14579, *Listeria innocua* ATCC 33090, and *Escherichia coli* ATCC 11229, was analysed using a well diffusion assay with 24-hour overnight cultures.

A total of 13 bacterial isolates were obtained, including two *Pediococcus pentosaceus*, two *Weissella confusa*, one *Weissella cibaria*, one *Leuconostoc mesenteroides*, and seven Leuconostoc citreum strains. The strongest pH reduction occurred with the two *W. confusa* strains, while the *P. pentosaceus* strains showed no pH reduction. On MRS agar with glucose and sucrose, *P. pentosaceus* displayed a ropy phenotype, whereas all other strains showed a mucoid phenotype on sucrose agar, indicating that all isolates were capable of EPS production. The well diffusion assay revealed that growth of the pathogens and surrogates was inhibited the most by *P. pentosaceus*.

The results underline the acidification ability of *W. confusa*, the antimicrobial activity of *P. pentosaceus*, and the EPS production capability of all isolates. Isolated strains show potential for improving the safety and texture of fermented pea protein.

3.24 Toxigenic potential of *Staphylococcus aureus* isolated from suspicious food in Germany

Sara Schaarschmidt, Sven Maurischat

German Federal Institute for Risk Assessment (BfR), Department Biological Safety, National Reference Laboratory for coagulase-positive staphylococci including *Staphylococcus aureus*, Berlin, Germany

The bacterium *Staphylococcus aureus* is a typical coloniser of human and animal skin and is also frequently found in the environment. It is a commensal bacterium with pathogenic potential and can exhibit a broad range of virulence factors. Among those are staphylococcal enterotoxins (SEs), which are produced by some strains at higher cell densities and under suitable conditions, such as those

found in many non-refrigerated food matrices. SEs are heat-stable superantigens with emetic potential and ingestion can cause foodborne disease at very low doses. So far, around 30 SE genes and SE-like genes have been identified; for the latter, the emetic action of the encoding protein has yet not been proven.

The National Reference Laboratory for coagulase-positive staphylococci including *Staphylococcus aureus* (NRL-Staph) supports the laboratories of the federal states in official food control and monitoring programmes, among other activities, through confirmation analysis and characterisation of isolates.

We present an overview on *S. aureus* isolated from suspicious foods during official food controls in Germany over the last 12 years, which were characterised at the NRL-Staph for their SE(-like) gene profiles by whole genome sequencing. Additional virulence factors, such as the toxic shock syndrome toxin (TSST) and the Panton–Valentine leucocidin (PVL) are also considered.

Acknowledgement: We thank our colleagues from the federal states laboratories and official food inspections.

3.25 Plant-based meat alternative products: a microbiological perspective

Gyde Joswig, Christina Böhnlein, Jan Kabisch, Charles M. A. P. Franz, Gregor Fiedler

Institut für Mikrobiologie und Biotechnologie, Max Rubner-Institut, 24103 Kiel

A new generation of vegetarian and vegan foods has increased in popularity for several years. This diverse and highly innovative product group is produced both by large companies with an established production infrastructure and by smaller companies such as start-ups.

Plant-based substitute products (plant-based meat alternatives) are amenable to microbiological growth due to their high water activity and their neutral to slightly acidic pH values, and should therefore be refrigerated. Due to the plant-based ingredients and production conditions the entry of bacterial spores, in particular Bacillus and Clostridium species which can survive the thermal processing of meat substitute products, is to be expected. Therefore, studies on the microbiological contamination of products and their ingredients were carried out to better understand the influence of ingredients, the manufacturing process and the shelf life (IGF project 23031 N).

A total of 30 plant-based meat alternatives were microbiologically tested and showed total viable counts between <100 to 5*107 CFU/g. Aerobic Bacillus spp. were detected at counts ranging from <10 to 2.9*103 CFU/g. B. cereus group bacteria and sulfite-reducing Clostridia were only detected in 4 and 2 products, respectively, both at low levels of 10-30 CFU/g.

The ingredients (spices and flavour mixes, plant proteins) specific to meat alternatives showed a total viable count of up to 3*105 CFU/g, aerobic *Bacillus* spp. of up to 2.5*105 CFU/g, *B. cereus* group of up to 110 CFU/g and sulfite-reducing *Clostridia* of up to 200 CFU/g.

End products were also stored beyond their expiry date at 10°C in their original packaging and analysed for spoilage after 14 and 28 days. Although large increases in total viable counts were observed for some products, counts of aerobic Bacillus spp. and sulfite-reducing Clostridia did not change over time. Consistent with this finding, very few psychrotrophic aerobic Bacillus spp. could be isolated from the samples.

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3.26 Shigatoxin-producing E. coli in buckwheat and other “uncommon” flours

Fatma Maar Matar,Frieda Jordan, Sebastian Steffan, Sharon Eddington, Carolin Hobe, Michaela Projahn,Elisabeth Schuh, Carolina Plaza-Rodriguez, André Goehler

German Federal Institute for Risk Assessment (BfR), Department of Biological Safety, Max-Dohrn Str. 8-10, 10589 Berlin, Germany

Shigatoxin-producing *E. coli* (STEC) are feared as food pathogens and can be isolated from flour and flour products. Current research focuses mainly on common flours such as wheat, rye and spelt flour. In 2020, approximately 9% of flour samples in Germany were found to be contaminated with STEC. However, data on other flours is not yet available. This study looks at buckwheat and other flours (corn, rice or green spelt) available in local supermarkets. Firstly, we aimed to determine the occurrence of STEC in 28 flour (20 buckwheat and 8 corn flour) samples and secondly aimed to unravel prevalent STEC (sero-)types. The presence of STEC were tested according to ISO/TS 13136:2012 (or §64 LFGB L00.00- 150(V)) and §64 LFGB L25.00 whereas modifications for optimization were compared. All samples were analysed in four times 25 g subsamples to increase the detection of STEC and other intestinal pathogenic E. coli. In addition to STEC detection, basic microbiological parameters were also determined for these samples. All eight corn flour samples were molecularly negative for Shiga toxin genes (*stx1* and *stx2*). In contrast, STEC was isolated from three of the 20 buckwheat flour samples. Additionally, five STEC strains, which were isolated from buckwheat samples within the framework of the German National Zoonosis Monitoring in 2024 by federal laboratories, were integrated into the analysis. Overall, five of the eight STEC isolates from buckwheat samples belong to serotype O187:H28 carrying *stx2g*. Whereas one sample contained a second STEC of serotype O103:H2 harbouring *stx1d*. The results indicate a contamination of buckwheat flour with similar STEC -types previously found in wheat and rye flour. However, the source of contamination and its distribution within the flour production/food chain remain unclear and require further investigation.

3.27 *Escherichia coli* - plating onto the surface of TBX agar - Validation study of a surface method according to DIN EN ISO 16140-2:2020-12 for the enumeration of β-glucoronidas positive Escherichia coli in all human food products, pet food and production environmental samples

Dietrich, J.[1,3], Porschhöfer, M.[1,3], Schneider, L.[2,3], Langen, M.[1,3], Kneißler, A.[2,3]

[1] Dr. Berns Laboratorium GmbH & Co. KG, Bendschenweg 36, 47506 Neukirchen-Vluyn, Deutschland
[2] Labor Kneißler GmbH & Co. KG, Unterer Mühlweg 10, 93133 Burglengenfeld, Deutschland
[3] Ariana Laboratories &Consulting Group, Ariana Holding GmbH, Unterer Mühlweg 10, 93133 Burglengenfeld, Deutschland

Industry Abstract

Regulation (EC) No 2073/2005 lays down microbiological criteria for foodstuffs. It requires businesses to carry out microbiological self-checks. These checks must verify, for example, compliance with process hygiene criteria for certain food groups for parameters such as *Escherichia coli*. In all cases, there are specific requirements for the test method to be used. In the case of *Escherichia coli*, the standard is DIN ISO 16649-2:2020-12 Microbiology of food and animal feeding stuffs - Horizontal method for the enumeration of beta-glucuronidase positive *Escherichia coli* - Part 2: Colony count at 44 °C using 5-bromo-4-chloro-3-indolyl-β-D-glucuronide. The application of this standard includes the use of the pour-plate method, which is very laborious for laboratories. However, Regulation (EC) No 2073/2005 allows alternatives to the listed reference methods. However, the use of such alternative methods requires a full validation against the reference method according to the requirements of DIN EN ISO 16140-2:2020-12.

In this study, an alternative method for the determination of β-glucuronidase positive *Escherichia coli* was developed and successfully validated against the reference method. In this method, a volume of diluted sample is applied to the surface of a TBX agar plate. Plating is performed manually with a spatula or logarithmically with a spiral plater. After incubation for 18-24 hours at +44 ± 1 °C, the typical blue to turquoise coloured colonies can be read.

Validation was performed on 7 categories (food, feed and environmental samples) with a total of 105 samples.

The working standard according to DIN EN ISO 16140-4:2020-12 includes two stages for validation:

Stage 1: Comparison of the alternative method with the reference method. For the method under validation, this includes: Investigation of relative precision, Precision profile approach and exclusivity/inclusivity study (30 non-target strains and 65 *Escherichia coli* strains).

Stage 2: Interlaboratory comparison of the alternative method with the reference method, performed in 4 different laboratories.

The data generated were evaluated according to DIN EN ISO 16140-2:2016-11. For all contamination levels and categories considered, the alternative method is recognised as equivalent to the reference method.

3.28 Rare Salmonella serovars found in sesame-based products in Germany from 2020 to 2024: Characterization of isolates and sequences from the NRL for Salmonella

Marina C. Lamparter[1], Maria Borowiak[2], Michael Pietsch[3], Carlus Deneke[2], Andreas J. Stroehlein[2], Istvan Szabo[1], Sandra Simon[3], Jennie Fischer[1]

[1] German Federal Institute for Risk Assessment (BfR), Department of Biological Safety, Unit Food Microbiology, Host-Pathogen-Interactions, National Reference Laboratory for Salmonella, Berlin, Germany
[2] German Federal Institute for Risk Assessment (BfR), Department of Biological Safety, Unit National Study Centre for Sequencing in Risk Assessment, Berlin, Germany
[3] Robert Koch Institute (RKI), Unit of Enteropathogenic Bacteria and Legionella / National Reference Centre for Salmonella and other bacterial enteric pathogens, Wernigerode, Germany

Salmonella spp. from imported sesame products have become a recurring issue in recent years in Germany.

Sesame paste has a long shelf life and is usually consumed without further bacterial inactivation steps in ready-to-eat products like hummus, but is also ingredient of sweets like halva or “Dubai chocolate”. The protective high-fat, low-aw matrix is supposed to reduce the Salmonella infectious dose. In contrast to well-known Salmonella outbreak vehicles like raw meat or eggs - often associated with the prominent TOP 2 serovars Enteritidis or Typhimurium - outbreaks associated with sesame-based products frequently involve otherwise rare serovars. Such partially multinational outbreaks comprising significant numbers of human cases have drawn attention to the combination of these uncommon serovars and plant-based food vehicles.

To get an overview of Salmonella serovars, isolated from sesame-based products in Germany, 109 Salmonella isolates from these matrices sent to the NRL for Salmonella from 2020 to 2024 were analyzed with classical serotyping methods and 61 of them were further analyzed with NGS technology and bioinformatic tools (ABC pipeline) for characterization and comparison. Additionally, 37 sequences of such products submitted by official state laboratories were included in the analysis. Sequences were compared to sequences of human isolates to find genetic matches.

Out of 109 isolates, 70 isolates came from tahini, 31 from halva, five from sesame, few from hummus and other sesame products. Together, 29 different serovars were typed by serological analysis, including three Agona and one Derby, but no other TOP serovars.

The 98 sequences/isolates were submitted by 13 different federal states; 60 were from tahini and mixed sauces, 34 halva, few from hummus, yogurt mix or sesame seed. From the sequences, 24 different serovars were identified. The cgMLST analysis revealed 16 single linkage clusters at threshold 10 AD, consisting of two to 16 sequences. The biggest cluster was S. Amsterdam with 16 sequences from 2020-2022 from tahini and halva from six states. In total, 90 sesame-product Salmonella sequences were compared to sequences from human cases at the RKI and identified matches to single isolates, but also to several human clusters, including one connected to an international multi-serovar outbreak associated with sesame-based products.

For safe tahini production, control of ingredient quality and roasting parameters is most important, but also contamination prevention after roasting. With sesame-based products and raw material being imported to Germany, international collaboration is essential to secure food safety of these products. This includes enhanced control and

monitoring efforts that target this issue. This assessment of Salmonella from sesame-based products available in Germany provide an improved perspective for further studies, such as trace-back investigations, which should help to optimize the safety of these products.

3.29 Microbiome profiling in the COPLANT study

Andreas Dötsch[1], Katharina Penczynski[2], Stefan Dietrich[2], Dirk Haller[3], Ina Danquah[4], Christine Dawczynski[5], Michael Leitzmann[6], Beate Fischer[6], Ute Nöthlings[7], Tilman Kühn[8], Maria Wakolbinger[8], Sandrine Louis[1], Benedikt Merz[1], Cornelia Weikert[2]

[1] Max Rubner-Institut
[2] German Federal Institute for Risk Assessment
[3] Technical University of Munich
[4] Heidelberg University
[5] Friedrich Schiller University Jena
[6] University of Regensburg
[7] Rheinische Friedrich Wilhelms University Bonn
[8] Medical University of Vienna

Despite the growing interest in plant-based diets and the increasing debate about their risks and benefits, recent studies investigating the associated health status or long-term health effects are scarce.

The planned prospective cohort COPLANT “COhort on PLANT-based diets” aims at assessing detailed data on nutrition, health and a collection of other relevant parameters to determine diet-specific differences. Baseline recruitment began between April and June 2024 and encompasses 7 study centers in Germany and Austria. A total of ~6,000 participants aged 18-69 years is planned to take part over 3 years. People who have been following a vegan, vegetarian, pescetarian or omnivorous diet for at least a year can take part.

All participants of the COPLANT study are asked for the collection of a cooled complete stool sample and/or a subsample with DNA stabilizer. Microbiota composition and diversity will be measured by 16S rRNA gene amplicon profiling, augmented by the determination of fecal short chain fatty acid and bile acid concentration. In a subgroup we will apply metagenomic shotgun sequencing to determine bacterial strain diversity and to functionally characterize microbiomes. The NutriDiary app and a detailed food frequency questionnaire encompassing plant-based food items is used to assess usual dietary intake. Furthermore, body composition, bone health, and nutritional behavior (e.g., meal preparation habits) are assessed. Blood and urine will be analyzed to evaluate nutritional status, metabolic markers, relevant contaminants and residues. In a subproject, two study centers will also assess oral and dental health and microbiome parameters.

By February 2025, 849 participants (= 14.1% of the aim) were included in the study, of which 761 (12.7 %) had provided full stool samples. Major challenges include the recruitment of pescetarians, especially males. To address this, target recruitment strategies are being explored, such as outreach via specialized communities.

3.30 The WHO Alliance for Food Safety: Strengthening global food microbiology through enhanced laboratory capacity and multisectoral collaboration

André Goehler[1], Tuyet Hoang[2], Carmen Joseph Savelli[3]

[1] German Federal Institute for Risk Assessment (BfR), Department Biological Safety, Berlin, Germany
[2] Microbiological Diagnostic Unit Public Health Laboratory, Peter Doherty Institute for Infection and Immunity, Melbourne, Australia
[3] Monitoring and Surveillance Unit, Nutrition and Food Safety Department, WHO, Geneva, Switzerland

The World Health Organization (WHO) established the WHO Alliance for Food Safety to collaboratively implement the WHO Global Strategy for Food Safety (2022–2030), with a focus on strengthening global foodborne disease (FBD) surveillance and food contamination monitoring. The Alliance fosters multisectoral collaboration, advocates for robust legal frameworks to support effective FBD surveillance, builds laboratory capacities, and facilitates the generation and exchange of high-quality data and best practices.

Within the Alliance, the Laboratory Capacity Building Working Group plays a central role in achieving these objectives. It aims to enhance national public health, animal health, and food analysis laboratories globally, taking a One Health approach. Within the broader field of food microbiology, laboratories focus on identifying and monitoring diverse microbial hazards, including bacteria, viruses, parasites, and toxins, that affect food safety. Comprehensive microbiological analysis and advanced characterization techniques are crucial for identifying contamination sources, understanding transmission pathways, and detecting emerging microbial threats. The Alliance supports countries in strengthening their laboratory capabilities, promoting standardized analytical methods, enabling effective data sharing, and encouraging global collaboration to mitigate microbiological risks throughout the food system.

By strengthening laboratory networks, enhancing surveillance infrastructures, and promoting best practices, the WHO Alliance for Food Safety aims to significantly reduce foodborne illnesses and promote safer food systems worldwide. This collaborative approach enables national authorities to more effectively prevent, detect, and respond to foodborne outbreaks, thereby protecting public health and reducing the global impact of foodborne pathogens.

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German Federal Institute for Risk Assessment (BfR)
Max-Dohrn-Straße 8-10
10589 Berlin, Germany
T +49 30 18412-0
F +49 30 18412-99099
bfr@bfr.bund.de
bfr.bund.de

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