

Training in metataxonomics-integrated risk assessment for foodborne pathogens in the Polish and Spanish dairy chain (DAIRYPOL)

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Abstract

Food safety is a key concern in the European Union, with the harmonisation of risk assessment methodologies being a strategic priority. The EU-FORA programme, coordinated by EFSA, promotes standardised approaches to microbial risk assessment across Europe. Metagenomic sequencing has advanced the understanding of microbial ecosystems in food production, with Illumina (short-reads) and Oxford Nanopore Technologies (ONT) (long-reads) playing significant roles in detecting pathogens and characterising microbial communities. These technologies differ in accuracy, read length and resolution, potentially influencing risk profiles when used in quantitative microbiological risk assessment (QMRA). This fellowship, conducted at UPCT in Spain and IBPRS-PIB in Poland, compares the two platforms in assessing microbial populations in Polish and Spanish dairy chains. It explores how sequencing strategy affects hazard identification and QMRA outcomes, while also demonstrating how metagenomic data can enhance predictive modelling and support practical improvements in food safety management.

KEYWORDS

dairy production chains, metataxonomic, quantitative microbial risk assessment, whole genome sequencing

CONTENTS

Abstract.....	1
Summary	3
1. Introduction	4
2. Background and Terms of Reference	4
3. Description of Work Programme	4
3.1. Aims	4
3.2. Activities.....	5
3.3. Laboratory experience.....	5
4. Data and Methodologies.....	5
4.1. Sampling in polish and Spanish dairy environments	5
4.2. Bioinformatics analyses and predictive modelling	5
4.3. Quantitative microbiological risk assessment (QMRA)	5
4.4. Methodological integration.....	6
4.5. Secondary scientific activities during the fellowship	6
5. Assessment	6
6. Conclusion	7
Abbreviations	7
Acknowledgements	7
Copyright for non-EFSA content.....	7
Disclaimer.....	7
References.....	7

SUMMARY

Food safety is a critical concern within the European Union, with the harmonisation of risk assessment methodologies being an important priority. The EU-FORA programme, coordinated by EFSA, aims to strengthen cooperation and promote the standardisation of food safety risk assessment practices across Europe. One of these areas of interest is the integration of metagenomic sequencing technologies into microbial risk assessment, transforming the understanding of microbial ecosystems in food production. Sequencing platforms such as Illumina and Oxford Nanopore Technologies have become essential tools for identifying pathogens, resistance genes, and microbial community dynamics. However, these platforms differ significantly in read length, accuracy, throughput and resolution, potentially introducing variability into microbial risk profiles, particularly when applied in quantitative microbiological risk assessment (QMRA).

This fellowship was developed jointly by UPCT in Spain and IBPRS-PIB in Poland, two institutions with extensive expertise in food safety and biotechnology. This technical report presents the work the fellow developed to establish a comparative evaluation of the Illumina and Oxford Nanopore sequencing platforms in analysing microbial populations across Polish and Spanish dairy production chains. It investigates how variations in sequencing strategies may impact the accuracy of microbial hazard identification and the outputs of downstream QMRA models. The findings will contribute to improved food safety management protocols in the dairy sector and beyond.

1 | INTRODUCTION

Advances in molecular microbiology have revolutionised the detection and characterisation of microbial communities in food environments. There are different sequencing approaches used in microbial analysis, each with distinct features and applications. Whole genome sequencing (WGS) provides the complete DNA sequence of an individual organism, allowing for precise identification, strain-level resolution and detection of virulence and antimicrobial resistance genes. It is highly informative but typically requires pure isolates. Metagenomic sequencing, or shotgun metagenomics, sequences all DNA present in a sample, facilitating taxonomic and functional characterisation of complex microbial communities without the need for culturing. While metagenomics is powerful for exploring microbiomes and detecting pathogens in mixed samples, it is more computationally demanding.

A third approach, 16S ribosomal RNA (rRNA) gene sequencing has become a frequent tool for identifying bacterial populations (metataxonomics) in complex matrices like food products and processing sites. This conserved genetic marker contains hypervariable regions facilitating identification at genus-level and, sometimes, species-level resolution. As a culture-independent method, it detects both culturable and non-culturable microorganisms, which is crucial for comprehensive food safety assessments.

16S rRNA sequencing remains a widely used (Johnson et al., 2019). In dairy production, it can help to effectively characterise the microbial composition of raw materials and processing environments. Dairy microbiota includes beneficial lactic acid bacteria, spoilage organisms and opportunistic pathogens (Quigley et al., 2012), all influencing product safety and quality. As genomic tools become routine in dairy safety monitoring, understanding the strengths and limitations of 16S sequencing is essential for protocol standardisation and improved risk assessments. This is especially relevant across countries like Spain and Poland, where variations in processing, hygiene and climate shape microbial profiles.

Despite its advantages, 16S rRNA sequencing has limitations. Short-read platforms like Illumina MiSeq usually target 1–2 variable regions (e.g. V3–V4), limiting resolution and risking misclassification of closely related taxa at species level. Long-read platforms like Oxford Nanopore Technologies (ONT) cover near full-length genes, improving resolution but introducing higher error rates and interpretation challenges (Ciuffreda et al., 2021). Additionally, DNA from dead cells may inflate risk estimates unless paired with viability assays (Emerson et al., 2017), one of the issues for accurate QMRA (Quantitative Microbiological Risk Assessment) exposure and risk characterisation. Thus, platform choice and methodological differences can significantly alter microbial community profiles and hazard identification outcomes.

QMRA provides a structured, science-based approach to estimate and manage microbial risks along the food chain. It includes hazard identification, exposure assessment, hazard and risk characterisation. Applied to spoilage (Koutsoumanis et al., 2021), QMRA helps the food sector to estimate the probability that a product becomes unacceptable due to microbial growth, helping with shelf-life decisions and reducing waste. Probabilistic models simulate spoilage under varying conditions and identify microbial control points. While traditionally used for pathogens, QMRA is increasingly applied to spoilage microorganisms, enhancing risk-based decision-making. Integrating molecular methods, especially metagenomics, improves precision in hazard identification, vital for estimating prevalence and assessing risk.

In this project, we investigate how the platform of choice (short-read Illumina or long-read ONT) affects the 16S-based pathogen hazard identification and QMRA results in Polish and Spanish dairy chains. Ultimately, this work will contribute to the development of a harmonised risk assessment across Europe.

2 | BACKGROUND AND TERMS OF REFERENCE

The European Food Risk Assessment Fellowship (EU-FORA) is a practical training program aimed at increasing the number of food safety risk assessment experts in Europe and promoting Member States' participation in risk assessment activities. The fellowship project, entitled Training in metagenomics-integrated risk assessment for foodborne pathogens in the Polish and Spanish dairy chain (DAIRYPOL), was developed through a partnership between the Institute of Agricultural and Food Biotechnology-State Research Institute (IBPRS-PIB)(Poland) as the sending institution of the fellow Dr. Joanna Bucka-Kolendo, and the Technical University of Cartagena (UPCT) (Spain) as the hosting institution, with Dr. Enriqueta Garcia-Gutierrez and Prof. Pablo S. Fernández as supervisors.

3 | DESCRIPTION OF WORK PROGRAMME

3.1 | Aims

The fellowship was a multidisciplinary, hands-on training combining theory and practice in food safety, bacterial genomics analyses and microbial risk assessment. Its goal was to examine how the choice of sequencing strategy (short-read Illumina vs. long-read Oxford Nanopore) can affect the microbial hazard detection in dairy environments and the resulting impact on QMRA outcomes.

3.2 | Activities

At the start of the fellowship, the fellow underwent training at the UPCT, focusing on microbiological risk assessment methodologies and software used by food safety authorities, industry and academia. The training covered databases, statistical tools for experimental design and data interpretation, and UPCT-developed web platforms such as bioinactivation, biogrowth and bioOED.

3.3 | Laboratory experience

Following the initial theoretical phase, the fellow conducted experimental work. Sampling was conducted in two factories, one in Murcia (Spain) and one in Warsaw (Poland). Swabs were collected on surfaces at different points in the cow farm and in the dairy-processing factories. Finally, final products from both companies were collected and analysed. Subsequently, DNA was extracted and sequenced, applying next-generation sequencing (NGS). The fellow expanded her expertise by using the Oxford Nanopore MinION, complementing prior experience with Illumina platforms. Sequencing microbial DNA from milk, dairy products and surfaces allowed her to assess how platform choice affects taxonomic resolution and microbial community profiling. These activities generated key data for predictive model development and deepened her understanding of how experimental design affects variability and uncertainty in microbial risk assessment.

The resulting data supported the construction and validation of predictive models describing microbial behaviour in the dairy chain. Using R and the bioinactivation, biogrowth and biorisk packages, both sequencing datasets were integrated to examine how platform-related differences impact growth predictions and spoilage risk estimates. This process also strengthened the fellow's skills in model development, selection, validation and the integration of uncertainty.

In the final phase, the fellow implemented a full QMRA for dairy production in Poland and Spain, defining scope, identifying contamination pathways and quantifying hazards using both experimental and public data. The models enabled comparative risk assessments, showing how regional differences in production, environment and hygiene affect consumer exposure and risk. This cross-country comparison helped contextualise findings within EU-wide food safety goals.

4 | DATA AND METHODOLOGIES

The fellowship combined experimental, bioinformatics and quantitative modelling approaches to assess how different WGS strategies affect microbial hazard identification and QMRA outcomes in the dairy production chain.

4.1 | Sampling in polish and Spanish dairy environments

The primary data consisted of microbial samples collected from raw milk, dairy products at various processing stages, and contact surfaces in farms and production facilities across Spain and Poland. These samples reflected typical environmental and product-related microbiota in dairy settings. DNA was extracted using standardised protocols optimised for dairy matrices and surfaces. Sequencing libraries were then prepared following Baker's protocols (<https://quadram.ac.uk/wp-content/uploads/2021/06/5.-16S-Illumina-Library-Preparation-Protocol-v1.0.pdf>) for two platforms: Illumina MiSeq (short-read, high-accuracy) and ONT MinION (long-read, portable). Using both platforms enabled comparison of taxonomic resolution and community composition biases.

4.2 | Bioinformatics analyses and predictive modelling

Sequencing data were processed using standard pipelines: Illumina reads via QIIME2 (DADA2, SILVA/Greengenes), ONT reads via MinKNOW, Guppy, EPI2ME and NanoCLUST. Special attention was given to ONT error correction and taxonomic resolution. Comparative analysis focused on community structure, diversity indices and detection of key pathogens and spoilage organisms, assessing cross-platform consistency.

The sequencing-derived microbial data were integrated into predictive models describing microbial behaviour in the dairy supply chain. Metataxonomics abundance data were translated into location mapping to evaluate the changes across the production lines.

4.3 | Quantitative microbiological risk assessment (QMRA)

The QMRA framework followed the Codex Alimentarius four-step process: hazard identification, exposure assessment, hazard characterisation and risk characterisation. Both deterministic and probabilistic methods were applied. Exposure models estimated microbial loads at consumption, accounting for contamination sources, processing/storage growth and surface cross-contamination. They included variability in storage times, temperatures and hygiene practices in Polish and

Spanish dairy chains. Risk characterisation results quantified consumer exposure and the probability of adverse health effects or spoilage.

4.4 | Methodological integration

This project highlights the strong influence of NGS strategy on microbial community detection and its downstream effects on QMRA. Combining both platforms may offer the most comprehensive QMRA outcomes. QMRA models should account for sequencing biases, and regulatory frameworks could benefit from hybrid approaches and cross-validation to ensure reliable food safety evaluations. Further innovation of the work programme was the integration of metagenomic data, traditionally used for descriptive microbial ecology, into predictive microbial risk models. This interdisciplinary approach will bridge the gap between molecular microbiology and quantitative food safety assessment, enabling a more nuanced understanding of how sequencing methodologies influence hazard identification and risk estimates.

4.5 | Secondary scientific activities during the fellowship

Together with the scheduled tasks, additional training and other scientific activities were provided by the hosting (UPCT) and sending (IBPRS–PIB) organisations, both in person and remotely. This improved the fellow's general knowledge of risk assessment and communication. Thus, the fellow was encouraged and supported to attend and gain experience from the following activities:

- The fellow attended an online Academy of the Manager organised by Horizon Europe Framework Programme (HE) National Contact Point for EU Research Programmes at the National Centre for Research and Development together with the network of Horizontal Contact Points on 18.9.2024–14.11.2024.
- A hands-on course on microbial risk assessment using R, enhancing proficiency in modelling tools used in food safety risk assessment, delivered by Dr. Alberto Garre (UPCT, Spain).
- Presentation of the 'Latest achievements and experiences in next-generation sequencing' at the Rapid Microbiology Testing School organised by Merck on 23.10.2024–24.10.2024.
- On-site workshop on Microbial risk assessment and use of Oxford Nanopore Technology at UPCT on 30.1.2025–31.1.2025.
- Visit of the headquarters of the Spanish Agency for Food Safety and Nutrition (AESAN) in Madrid and the National Centre for Food Laboratory, on 18.2.2025–19.2.2025. During the visit, the fellow benefited from AESAN's presentation on risk assessment, management and communication.
- On-site Mycology training – microscopy and bioinformatics on 7.3.2025 and 29.3.2025, organised at Warsaw University, Poland.
- On-site Illumina NGS workshop on 28.4.2025, conducted by David Baker (Quadram Institute Bioscience) at IBPRS-PIB.
- The results from the project are planned to be presented as a poster at the Joint International Conference of MIKROBIOKOSMOS & Central and East Europe Symposium on Microbial Ecology, which will take place on 22–24 September 2025 in Thessaloniki, Greece.

5 | ASSESSMENT

This fellowship project investigated how various NGS strategies might impact microbial hazard identification and QMRA outcomes in the dairy sector. Comparing short-read Illumina and long-read ONT data showed that platform choice strongly shapes our understanding of microbial communities along the food chain. Illumina provided high accuracy and genus-level resolution for dominant taxa, confirming its value in food microbiology. ONT, despite higher error rates, captured broader microbial diversity, detecting taxa missed by Illumina. However, ONT data requires careful error correction and validation before informing food safety decisions.

These differences affected hazard identification, contamination estimates and exposure assessments. Predictive models developed during the fellowship, revealed that microbial risk estimates varied by platform. Spoilage organisms and opportunistic pathogens found only in ONT data influenced initial contamination assumptions, sometimes increasing risk levels.

QMRA models for Poland and Spain revealed both common and region-specific risks. While critical control points were similar, differences in microbial prevalence, contamination sources and storage conditions led to distinct profiles. This highlights the need to adapt risk assessments to local contexts and supports harmonised and flexible tools across Europe.

The fellow actively participated in both institutions, joining meetings, seminars and collaborations, supporting professional development and building connections in the European food safety and microbiology community. Practically, the project provides useful insights for food safety authorities and the dairy industry. It supports development of more robust QMRA models, improving hazard detection, shelf-life prediction and food safety strategies.

6 | CONCLUSION

In conclusion, this fellowship will contribute to strengthened European food safety capabilities by integrating genomic identification of bacterial communities and quantitative risk modelling. The collaborative work between UPCT and IBPRS-PIB is an example of the value of cross-border partnerships in addressing complex food safety challenges. By training future risk assessors in cutting-edge analytical and modelling techniques, the programme supports EFSA's goal of harmonising risk assessment practices and enhancing food safety across the EU.

This study highlights the critical influence of the NGS strategy on microbial community detection and its downstream impact on QMRA models. The choice between Illumina and Oxford Nanopore affects not only the microbial diversity captured but also functional gene predictions and ultimately risk estimates. Combining data from both platforms may yield the most comprehensive QMRA insights. Differences in microbial compositions between Polish and Spanish dairy products also underline the need for region-specific risk assessments. QMRA models should account for sequencing biases, and regulatory frameworks may benefit from hybrid sequencing and cross-validation methods to ensure reliable food safety assessments.

ABBREVIATIONS

EU-FORA	European Food Risk Assessment Fellowship Programme
IBPRS-PIB	Institute of Agricultural and Food Biotechnology-State Research Institute
NGS	next-generation sequencing
ONT	Oxford Nanopore Technologies
QMRA	quantitative microbiological risk assessment
rRNA	ribosomal
UPCT	Polytechnic University of Cartagena
WGS	whole genome sequencing

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