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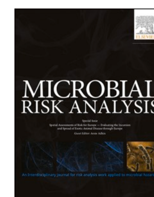

Next generation risk assessment: A proof of concept for the integration of genomic data on cold tolerance into quantitative microbial risk assessment for *Campylobacter jejuni* in poultry meat

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A B S T R A C T

Quantitative Microbiological Risk assessment (QMRA) models are essential tools for setting up mitigation strategies. Traditional QMRA modelling approaches do not account for the correlation between genetic traits and variability among pathogens, potentially leading to over- or underestimation of microbial exposure and associated risks. We aimed to integrate genomic data into QMRA to propagate bacterial strain variability and update the existing framework of QMRA, following a Next Generation Risk Assessment (NGRA) approach. We used a benchmark QMRA model describing the prevalence and concentration of *Campylobacter jejuni* on chicken in all stages from farm-to-fork, to model the risk of infection and illness related to consumption of chicken meat. We integrated extended the storage step, to account for genetic variability in cold inactivation by incorporating gene-level genomic data associated with cold tolerance, derived from literature and a large *C. jejuni* genomic dataset, into the traditional QMRA model by setting up cold inactivation curves from existing data to map the relationship between the number of cold tolerance genes and temperature-dependent inactivation. The predicted number of cases was 8822 human cases/year in the benchmark QMRA model. The contamination of meat with *C. jejuni* strains having lower cold tolerance genes can reduce the expected number of human campylobacteriosis cases up to 100%; on the other hand, higher number of cold tolerance genes resulted in an increase up to 335.8% on the expected number of cases. Although our results are based on simulations, we show a potential implementation of the genetic information into QMRA, linking risk estimates with whole-genome sequencing data. More research is needed to understand how genetic features shape phenotypical characteristics, which is one of the main uncertainties in the current NGRA model, and to further explore the implications for risk management.

1. Introduction

Quantitative microbiological risk assessment (QMRA) is a science-based process aimed at estimating health risks, including the variability and uncertainty associated with those risks. QMRA forms the scientific basis of risk analysis and is widely employed to support decision-makers by offering an objective and transparent evaluation of health risks and their underlying causes at a specific point in time (WHO, 2021). QMRA models can assess the effectiveness of mitigation measures and, with that, support the development of policies to prevent infections in both animals and humans. It can address a range of critical questions, such as the risk of food and water contamination (Teunis and Schijven, 2018), identifying or attributing sources of infection across populations, and prioritizing actions in public health (Pires et al., 2024), or helping pinpoint high-risk hazard species or husbandry practices that contribute to disease spread (Pires et al., 2018).

In general, QMRA is based on assessing exposure pathways using hazard's information available at the genus/species. However, most

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QMRA approaches neglect the correlation between strain variability and genomics, such as within-species heterogeneity in microbial virulence, host adaptation, growth/survival potential, and stress tolerance, which may result in over- or underestimation of microbial exposure and associated risk (Arnaboldi et al., 2023; Franz et al., 2016). Incorporating genomic data into QMRA could thus introduce more biologically relevant heterogeneity and enable the refinement of risk estimates, such as the risk of a certain outcome or the disease severity.

The incorporation of higher resolution omics data at the pathogen level, such as sequence data, into QMRA models is termed here Next Generation Risk Assessment (NGRA) (Arnaboldi et al., 2023; Cocolin et al., 2018; Collineau et al., 2019; den Besten et al., 2018; Franz et al., 2016; Haddad et al., 2018; Koutsoumanis et al., 2019; Rantsiou et al., 2018). Given that the link between the genetic marker and phenotypical characteristics is known, the NGRA could lead to a better risk estimation of, e.g., the number of human cases or health and transmission effects. This will contribute to an improved design of surveillance, control measures and intervention strategies.

Although developments in diagnostics and omics provide a wealth of information about pathogens and sequence data is nowadays increasingly available due to decreasing costs for analyses (Carbo et al., 2023; Wetterstrand, 2023), this information is not yet fully integrated within QMRA. Currently, the inclusion of sequence data within QMRA models focuses on foodborne hazards, like *Salmonella* and *Listeria monocytogenes*. Available examples suggest a more accurate risk prediction, concluding that the minority *Salmonella* and L. *monocytogenes* variants are responsible for most human infections (Fritsch et al., 2018; Godínez-Oviedo et al., 2022; Njage et al., 2020). In addition, the integration of sequence data in QMRA also showed the potential to differentiate the number of human cases caused by Shiga toxin-producing *Escherichia coli* infection by health outcomes (Njage et al., 2019).

Various QMRA models have been developed for *Campylobacter* (Nauta et al., 2009), which is considered a major bacterial cause of human gastroenteritis worldwide, and the most common bacterial cause of foodborne diseases. The QMRA models' estimates served as an input to establish a process hygiene criterion (PHC), impacting surveillance in the poultry sector, with poultry being the most important source of human campylobacteriosis (Thystrup et al., 2025). These models, as well as other models relevant to diverse pathogens, would benefit from refinements using existing sequence data, which will lead to the development of NGRA methods.

Previous models did not integrate genomic determinants of cold tolerance. This study aimed to provide proof of concept for the extension of an existing QMRA model for *Campylobacter jejuni* (Nauta et al., 2005) by incorporating available genomic data and comparing the outcome of the resulting NGRA with traditional QMRA. We describe the identification of genetic markers related to the cold resistance genes during the storage of poultry filets, and its application into one step of the QMRA, namely the cold storage step. We describe also the rationale behind incorporating the new parameters into a traditional *C. jejuni* QMRA model transforming it into a NGRA model.

2. Methods

2.1. Conceptual NGRA model for Campylobacter in poultry meat

The model framework for NGRA is based on the QMRA model developed within the CARMA project (Nauta et al., 2005) called here as classic QMRA. This model aimed to simulate the transmission of *C. jejuni* (hereafter called *Campylobacter*) through the food production chain by applying the 'Modular Process Risk Modelling' methodology (Lindqvist et al., 2002; Nauta, 2005, 2002). In a nutshell, it simulates the transmission of *Campylobacter* from 'farm to fork', starting with the entrance of a flock of live broilers into the slaughterhouse until the preparation of a filet at home, possibly contaminating a portion of salad to be consumed. The pathway is split up into some major consecutive: i) Processing (slaughter and industrial processing of the carcass), ii) Cutting (from the carcass to a skinned chicken breast filet), iii) Storage (before and at retail, and by the consumer), and iv) consumer phase (preparation and consumption).

NGRA uses *a priori* knowledge about the relevant genetic markers for a given risk pathway/exposure, as part of the hazard identification in classic QMRA. In this step, researchers describe the current state of knowledge about the phenomenon under study and the genetic features that drive the process. As revised by other authors (Arnaboldi et al., 2023) several properties of *Campylobacter* through the farm-to-fork QMRA are relevant for public health risks and vary among the different strains. Thus, these could be explored as points to integrate genomic data into classic QMRA. Those properties include survival and inactivation of *Campylobacter* under stressful conditions, such as high and low temperatures, during processing and storage of meat. During these steps, *Campylobacter* is exposed to a range of temperatures, and genetically adapted strains might survive better previously being exposed to these environmental conditions (Murphy et al., 2006).

To determine our focus on NGRA implementation, we first brainstormed with risk assessors and microbiologists to list the bacterial characteristics that could be modelled and would influence the risk throughout the risk pathway. Three characteristics were highlighted, namely cold tolerance, heat tolerance, and bacterial adherence to surfaces. Thereafter, we searched the literature for available candidate genes. The correlation between genetic markers and heat tolerance or microbial adherence was not clearly described in the available literature, but cold tolerant genes (CTGs) were better described (Hur et al., 2022). Therefore, the focus was on the cold tolerance during the storage step of chicken filets; we chose to include the effect of the number of cold tolerance genes (CTGs) on the cold tolerance in the classic QMRA model (see Section 2.3 for more details). Thus, in the current study, we focus on the relationships between the number of CTGs and the expected *Campylobacter* phenotypes that can potentially influence the survival during chicken filet storage and potentially drive the risk characterisation (Fig. 1).

2.2. Cold tolerance genes selection

A recent study tested cold tolerance in 79 *C. jejuni* strains isolated from retail raw chicken meat and discovered a set of 58 genes that were present in cold stress-resistant isolates, but absent in the cold stress-sensitive strains (Hur et al., 2022). This set was used as the representative set of genes linked to cold tolerance in our model, as we assume they give a benefit for surviving cold storage. The 79 *C. jejuni* strains could be classified in three categories based on phenotypic tests and genetic clustering: tolerant, intermediate and sensitive, respectively Cluster 4, Cluster 1 and 3 combined and Cluster 2 as defined by Hur et al. (2022).

To assess whether *C. jejuni* genomes contained the CTGs an ABRicate database was built named *C. jejuni* Cold Tolerance (CjColT), following the authors' instructions available on https://github.com/tseemann/abricate (Seemann, 2025), using FASTA files derived from *C. jejuni* subsp. jejuni NCTC 11,168 (GenBank accession: GCA_000009085.1) by extracting them with Artemis (version 17.0.1) (Rutherford et al., 2000), as no accession numbers were available but locus tags only. To test our assumptions on the number of cold tolerant genes within certain groups and hosts and to generate parameters necessary for modelling cold tolerance, like minimum, maximum and average number of genes, these genes were searched with ABRicate v. 1.0.1., using default settings, within the 79 genomes from the (Hur et al., 2022) study and the *C. jejuni* dataset from the DEPICT project (Mughini-Gras et al., 2021). This dataset contains 1060 *C. jejuni* genomes: 272 from patients with gastroenteritis, 66 surface water samples, and 689 animal samples. Within the animal samples, there are 186 broiler samples primarily from meat, 55 layer chicken samples primarily from faeces, 37 turkey meat samples, and 30 wild bird samples that are of interest for this study.

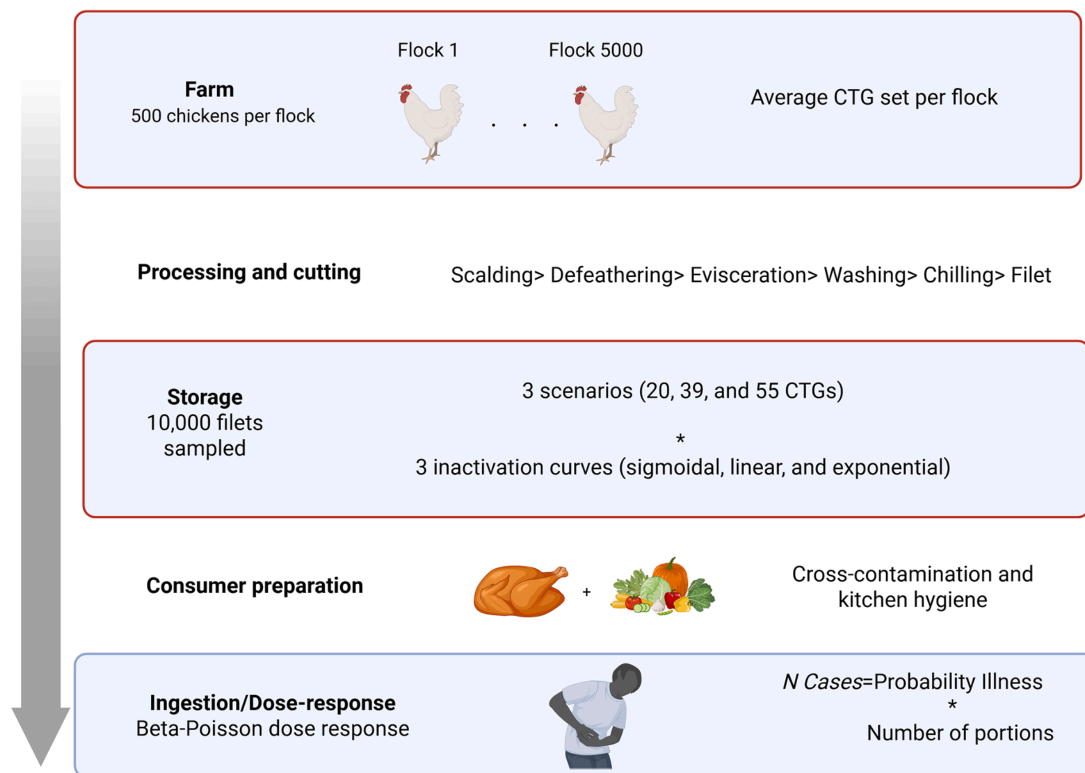

Fig. 1. Risk pathway used for the next generation risk assessment (NGRA) of chicken filets contamination by *Campylobacter*. CTG=cold tolerance genes. Red boxes indicate implementation of cold tolerance genes, first on the chicken carriage and later on the inactivation curve during storage.

Combining these animal samples with the samples from patients and surface water resulted in a set of 646 whole genome assembled datasets used in this study. Parameters for the model were derived based on the presence-absence tables created from the ABRicate output.

2.3. Model adaptation

As mentioned in Section 2.1, the classic QMRA model was used as a benchmark model to simulate the transmission of *Campylobacter* through the food production chain. We introduced a stochastic node at the start of the processing phase, assigning each of the 500 chickens (*i*) from flock (*j*) a specific number of CTGs $y_{i,j} \sim Poisson(\lambda_j)$, where λ_j is the average number of CTGs in the flock *j*, i.e., the model used the average number of CTGs for the *Campylobacter* population circulating among the flocks. The choice for propagating within-flock strain variability was based on the reports that although there is a dominant strain, still there is a substantial genetic variation reported on *Campylobacter* within broiler flocks (Damjanova et al., 2011; Vidal et al., 2016). The Poisson distribution was chosen because it is a natural choice when describing independent counts with a fixed average. The subsequent steps accounted for the contamination and cross-contamination during carcass dressing activities, following the same input parameter values and model as the original implementation. Also, similarly to the original model, we used the contamination on filets after partitioning as input to the storage step.

As input for the storage, 10,000 chicken filets were randomly sampled after partitioning. The classic QMRA model uses a stochastic node to select the $\log_{10}$ reduction (called $r_{storage}$) from a Pert distribution, approaching on average, approximately 1 $\log_{10}$ reduction on filets at 4°C for seven days. Here, we used several assumptions to link the parameter $r_{storage}$ with the number of CTGs (i.e., quantity $y_{i,j}$ explained before).

To match the number of CTG with a $\log_{10}$ reduction effect we assumed that the cold tolerance is a trait determined by a cumulative effect of the selected genes. A gene present in the genome is a functional gene, thus any changes within a gene (SNPs, indels) or its regulation are not included in this model. Every flock has one "dominant" *Campylobacter* strain carrying a certain number of CTGs, which means that for the same flock, only one average number of CTGs will be used for simulations. The number of CTG is not altered by the previous processing steps, such as scalding and chilling. The median number of cold tolerance genes observed in broiler meat samples from the DEPICT project (i.e., 39, see the results section for more details) corresponds to the average log reduction used in the classic QMRA implementation: $r_{storage} \sim 1\log_{10}$. Based on the information from 646 isolates from DEPICT project we assumed that the number of CTGs present in a *Campylobacter* strain range from minimum 20 to maximum 55. The relationship between the number of CTGs and $r_{storage}$ is assumed to be monotonic and decreasing, meaning that an increase in the number of CTG always results in a reduction of the $\log_{10}$ inactivation (Figures available in supplementary material 1).

We tested three different cold inactivation curves, and the parameters are available in Table 1. A sigmoidal curve: given by $r_{storage} = \frac{L1}{(1+e^{-b1*(y-c1)})}$, where y is the number of cold tolerance genes; L1 is the $r_{storage}$ value when CTG approaches zero; c1 is the inflection point of the curve at the x axis (i.e., the number of CTGs where the curve reaches the "middle point" or concavity change); and b1 is the $\log_{10}$ reduction rate per tolerance gene. In summary, upon adding more cold tolerance genes, the log reduction decreases in a specific pattern that starts slow, speeds up, and then slows down again, forming an S-shaped curve.

A linear curve: given by $r_{storage} = L2 - b2 * y$, where y is the number of cold tolerance genes; L2 is the intercept of the model i.e., the value of $r_{storage}$ when CTG equals zero; and b2 is the slope. The linear model is completely characterised by only two coordinate pairs. In our case, we set the slope to return approximately 1 $\log_{10}$ at 39 CTG using 4 $\log_{10}$ reduction as the intercept.

An exponential curve: given by $r_{storage} = L3 * c1^{-b1*y}$, where y is the number of cold tolerance genes; L3 is the $r_{storage}$ value when CTG ap-

Table 1

Parameters used to map the number of cold tolerance genes (CTG) into $\log_{10}$ cfu *Campylobacter* in broiler filets during storage for the three cold inactivation curves used. The calculated values of $r_{storage}$ for each curve are available in supplementary material 1.

Parameter*	Inactivation curve	Value (unit)
Sigmoidal		
L1	The maximum value for $\log_{10}$ reduction observed when the number of cold genes tolerance approaches zero by (Hur et al., 2022).	4 ($\log_{10}$ cfu)
c1	Inflection point of the curve at the y axis (i.e., at each cold tolerance genes de curve reaches the "middle point" or concavity change).	37 (CTG)
b1	Reduction rate on $\log_{10}$ reduction, translating how steep the curve is.	−0.52 ($\log_{10}$ cfu/CTG)
Linear		
L2	The maximum value for $\log_{10}$ reduction observed when the number of cold genes tolerance equals zero by (Hur et al., 2022).	4 ($\log_{10}$ cfu)
b2	Slope of the linear curve.	0.066 ($\log_{10}$ cfu/CTG)
Exponential		
L3	The maximum value for $\log_{10}$ reduction observed when the number of cold genes tolerance approaches zero by (Hur et al., 2022).	4 ($\log_{10}$ cfu)
c2	Exponent base.	1 dimensionless
b3	Reduction rate on $\log_{10}$ reduction, translating how steep the curve is.	0.88 ($\log_{10}$ CTG)

*Parameters for the sigmoidal and exponential curves were assessed to fit CTGs: (0, 39, 55) into the $\log_{10}$ reduction: (4, 1, 0) minimizing the sum of squared errors (SSE) between observed and predicted values, assuming normally distributed residuals. Optimization was done using the "L-BFGS-B" algorithm implemented as in R function "optim". For the linear curve the values were assessed to have a linear function with intercept equals 4 and $r_{storage} \sim 1$ when CTGs equals 39.

proaches zero; b1 is the rate parameter; and c1 is the exponent base.

2.4. Consumer phase model

For the start of the consumer phase model, the output from the storage i.e., 10,000 chicken filets, were sampled and submitted to domestic preparation and consumption. The consumer phase followed the same approach as in classic QMRA model, describing cross-contamination from raw chicken filets to hands, cutting board, water tap, and salad, assuming that the chicken meat is well cooked, and all *Campylobacter* on the meat is killed off, and the cross-contamination to the salad, which is consumed raw, is therefore the only relevant route for human exposure. The probability of illness, calculated using a Beta Poisson dose-response model (Teunis et al., 1999), is averaged over 10, 000 meals and multiplied by the number of portions consumed by young and adults in one year, to obtain the expected number of campylobacteriosis cases in humans. Here, we used the same parameter inputs as used originally in the classic QMRA model.

2.5. Model scenarios and simulation setup

We have set three different values for the within-flock average number of CTGs. Using the nomenclature in Section 2.3, we selected $\lambda_j = (20, 39, \textit{and } 55)$, perceived here as scenarios of low, medium, and high within-flock presence of CTGs, respectively. Those three scenarios are further combined with the three different cold inactivation curves (sigmoidal, linear and exponential) in a six-factorial scenario setting.

This was based on the information from 646 isolates from the DEPICT project, in which the median number of CTGs observed in broiler meat samples was 39, and the minimum 20 and a maximum 55, respectively. It is further assumed that the mean number of CTG is constant over the 5,000 flocks in the same scenario, and no between-flock variability is implemented for the CTGs. The within-flock variations propagate the between-chickens variability within the same flock. That goes in line with the CARMA model that propagates the between-chicken variability until the consumer phase. For each scenario, 5,000 flocks with 500 chickens generating 500 filets were simulated. The scripts we implemented in R (R Core Team, 2023) were based on scripts written by the German Federal Institute for Risk Assessment to translate the original CARMA implementation in @Risk to R (Correia Carreira et al., 2023), available on: https://git.wur.nl/wbvr_epi/ngra.git.

3. Results

3.1. Genomic parameters

In order to parameterize the later models correctly, we evaluated the number of cold tolerance genes predicted with the CjColT database and what their upper and lower thresholds are by screening the Hur et al. (2022) dataset of 79 isolates. Based on the phenotypic tests and genetic clustering they run, four clusters were defined of which one had a high tolerance to cold and one was very sensitive. The two remaining clusters both showed very similar intermediate cold tolerance; therefore, we classified these isolates in three cold tolerance categories: tolerant, intermediate and sensitive. The number of CTG in the tolerant cluster ranged from 57 to 47 genes, with the 47 being a clear outlier with all others having above 56 cold tolerance genes (Fig. 2A and Supplementary material 2). The number of CTG in the intermediate and sensitive clusters ranged from 49 to 33 and 17 to 15 genes, respectively.

Similarly, we described the distribution of cold tolerance genes per source within the selection obtained from the DEPICT data set considering several host sources. We observed similar CTGs averages for humans and broilers, and a lower number of CTGs in wild birds and surface water (Fig. 2B and Supplementary material 2). Moreover, the distribution of cold tolerance genes per isolate from broilers and humans has two bottlenecks that roughly align with the boundaries between the three sensitivity categories.

3.2. Integration of the genomic parameters into QMRA

First, for comparison purposes, we compare the results of the classic QMRA and the NGRA implementation from the "input" until the "chilling" step at the slaughterhouse. Notice that the models are the same during the processing steps. In the classic QMRA model, the authors report *Campylobacter* concentration of 6.7 and 4.3 log cfu/carcass in the input and chilling step, respectively. For the NGRA model simulations, the concentration was 6.8 and 4.4 in the input and chilling steps, respectively. (Fig. 3).

With the classic QMRA model, it was observed an average contamination on chicken filets, after storage, of 2.7 $\log_{10}$ cfu. This is similar to the observed value in NGRA using 39 CTGs for the sigmoidal cold inactivation model (2.3 $\log_{10}$ cfu). The average contamination on chicken filet using the linear and exponential models were 1.6, and 3.0 $\log_{10}$ cfu, respectively, at 39 CTGs. Also, the chicken filet contamination is higher with larger numbers of CTGs for all cold inactivation models (Fig. 4 and Supplementary material 1, Figure S1).

The classic QMRA model estimated 8822 cases of human Campylobacteriosis in one year. The NGRA scenarios using 20 CTGs decreased the number of cases in all three inactivation curves, approaching 100 % reuction in the sigmoidal inactivation curve. When using 39 CTGs increased the number of cases to 10,170 (by 15 %, and reduced to 3079 (−65 %) in the linear inactivation curve. The increase in the number of cases is more proeminent in the scenario with 55 CTGs reaching 335.8 % increase in the sigmoidal inactivation curve (Fig. 5).

4. Discussion

In this study, we proposed an extension to the classic QMRA for

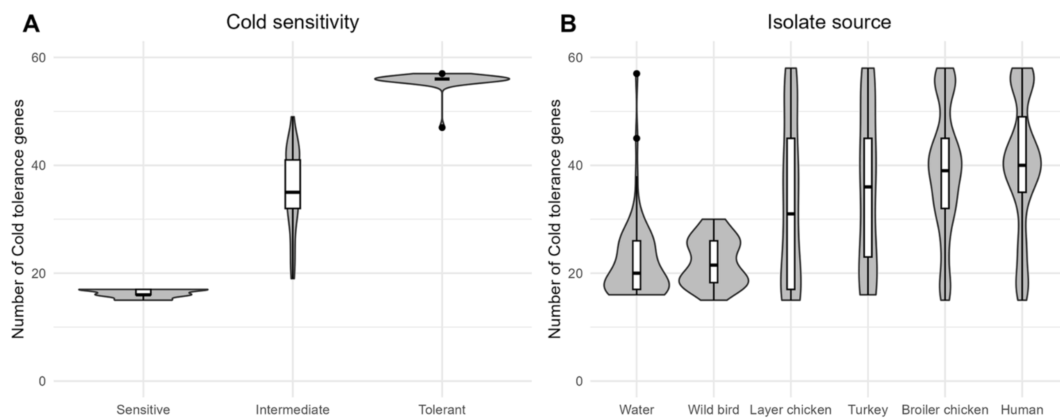

Fig. 2. Distribution of cold tolerance genes (CTGs): (A) per cold sensitivity category within the Hur et al. (2022) data set and (B) in six different sources of isolates from a selection of the DEPICT data set.

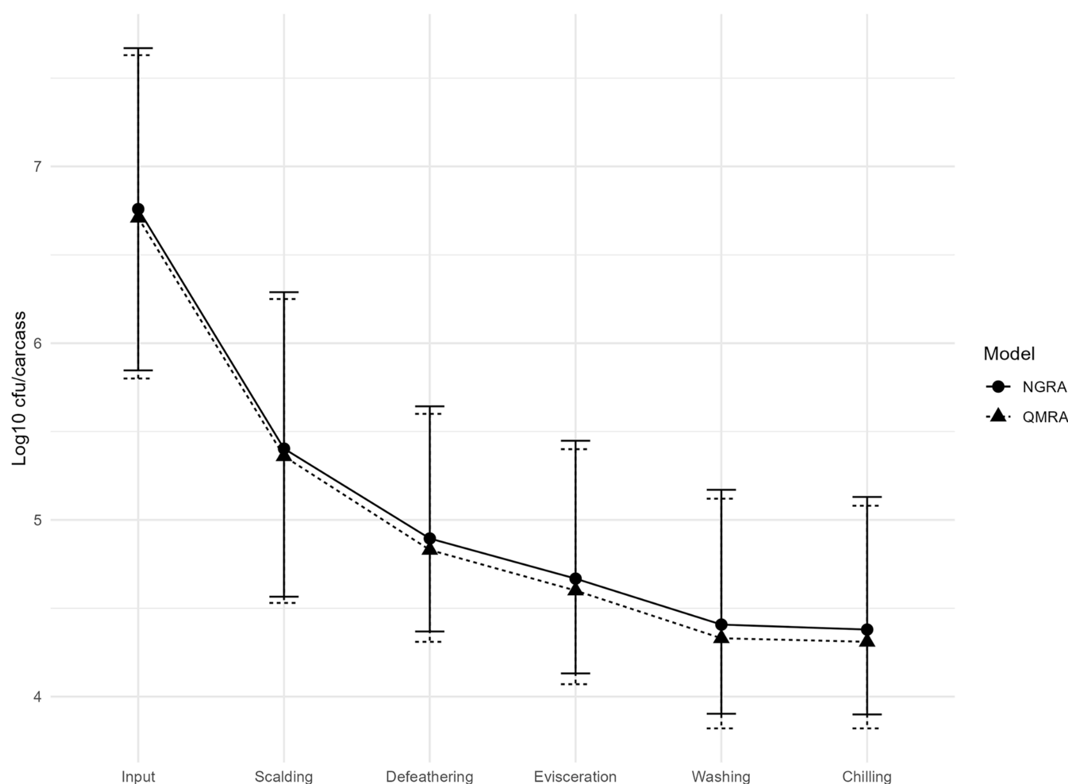

Fig. 3. Comparison between the average contamination of carcasses throughout the processing steps between the classic QMRA (dotted line with triangles) and NGRA (straight line with circles) models. The data of the classic QMRA results were extracted from the RIVM report 250,911,006, page 78, (Nauta M. et al., 2005).

Campylobacter (Nauta et al., 2005) by incorporating available genomic data of *Campylobacter* regarding the cold tolerance during the storage step. We described the assumptions and the rationale behind incorporating new parameters into a *Campylobacter* QMRA, which we now call NGRA. In the classic QMRA model, the inactivation was modelled using a $\log_{10}$ reduction following a Pert distribution, which leads to an “average cold inactivation curve”. Now, the parametrisation of the cold inactivation propagates the variation in *Campylobacter* strains observed in the available literature and genetic data (Fig. 1). We provide and theoretical exploration of three different curves mapping the cold inactivation as a function of the number of cold tolerance genes in combination with three different scenarios for the average CTGs present in flocks and how this affects the risk characterisation.

Before incorporating the expected effect of CTGs into the classic QMRA model, we evaluated if the number of CTGs differ among the three cold sensitivity categories, that were defined based on phenotypic cold tolerance and phylogenetic clustering, and whether there was a difference in CTGs between isolates from different sources. The distribution of CTGs did not overlaps for the sensitive, intermediate and tolerant phenotypes (see Fig. 2A). Moreover, the distribution of CTGs in broiler and human samples also showed some stratification. This indicates that the different cold tolerance phenotypes are different concerning CTGs and supports our choice for looking at three scenarios within the NGRA model. Further, we found that the closer you get to the production chain and human consumption, the more CTGs you find within the *Campylobacter* isolates, with the lowest numbers in water and wild birds and the highest in broilers and humans (see Fig. 2B). The very similar averages for humans (40.1 CTGs) and broilers (39.1 CTGs)

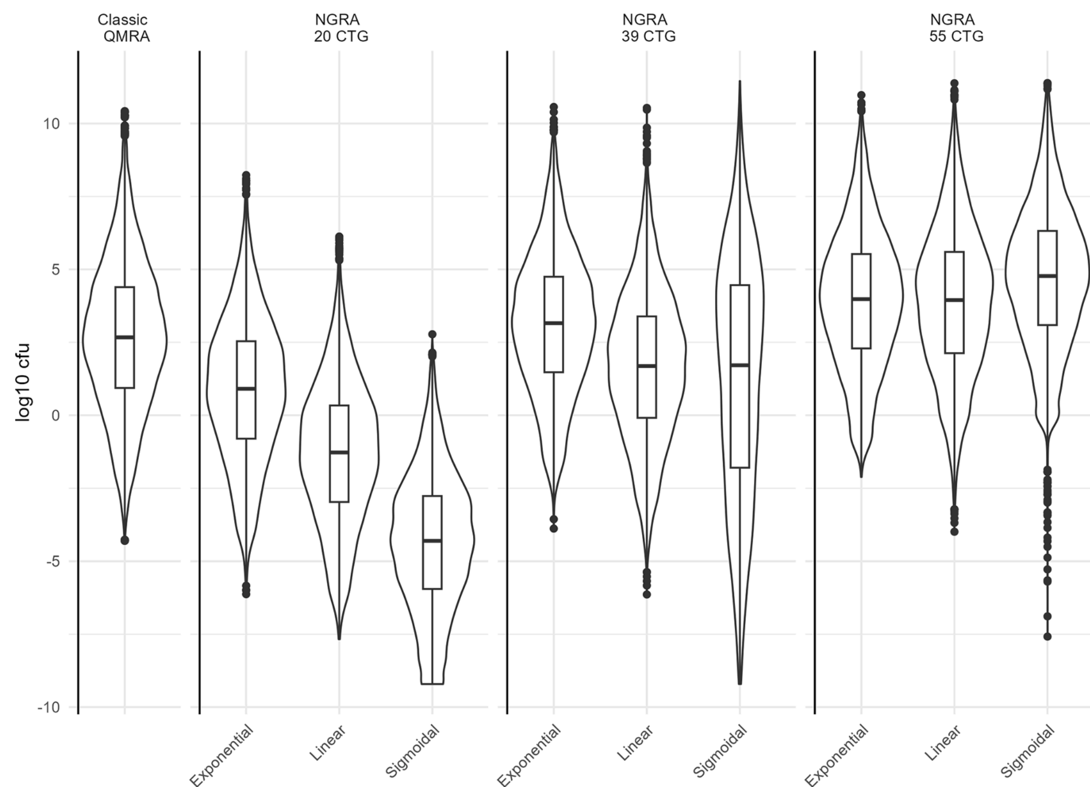

Fig. 4. Distribution of the 10,000 filet contamination comparing results of the classic QMRA model and the NGRA model, where different combinations of cold inactivation models (sigmoidal, linear, and exponential) and different next generation risk assessment model scenarios: 20, 39, and 55 cold tolerance genes (CTGs) were evaluated.

suggest that these sources are tightly linked, which is in line with the current notion that poultry meat is the main source of human campylobacteriosis (Mughini-Gras et al., 2021, 2012; Rosner et al., 2017), and the discussion from that isolates having a higher number of CTGs will more likely cause a human infection. The mechanism behind this proximity between the CTGs averages between humans and broilers could be hypothesized given a selective pressure throughout the production chain, leading, on average, strains with more CTGs to survive and be sampled in broiler meat and also expose humans. However, further research is needed to confirm this hypothesis.

In general, the classic QMRA and the NGRA QMRAs behave fairly similar in the slaughterhouse module. As explored in Fig. 3, the NGRA gives a slightly higher carcass contamination score, but still, this difference at the chilling step is 0.11 log_{10}cfu (i.e., ~1.28 cfu). During the consumer phase, we can observe similar contamination distributions on filets between the classic QMRA and the NGRA when we set the number of CTGs to 39. This is expected and happens because we set 39 CTGs to be the number of CTGs that leads to approximately 1 log_{10} reduction during the storage, which is the same average log^{10} reduction used for the classic QMRA model. The implementation of this model is robust enough to allow changes in the parametrization. For instance, if the interest is to explore the uncertainty around the average CTGs one could choose to propagate this uncertainty between the flocks using a stochastic distribution.

It can be seen that both the number of CTGs and the inactivation curve can change the number of campylobacteriosis cases. At 20 CTGs, the sigmoidal has the highest bacterial reduction (Fig. 4), leading to zero cases (Fig. 5). This is expected given the large shoulder that the sigmoidal curve has. On the other hand, the exponential curve quickly decreases the log_{10} reduction at 20 CTGs (see Supplementary material 1), leading to a larger number of cases compared to the other curves withing the same CTGs number. At 55 CTGs, the low and large tail of the sigmoidal model result in slightly higher median contamination compared with the exponential and linear models (Fig. 4). It is interesting to see that the number of cases in the sigmoidal model at 55 CTGs is much higher than in the linear and the exponential models. Those results reflect the complex relationship between the exposure dose and the number of cases caused by the dose-response model. Thus, small difference in contamination can lead to larger risk differences, highlighting the need for a better understanding of how the genetic features affect the survival of bacteria and, consequently, the risk.

Also, it is important to highlight that we propose a theoretical exercise making use of several knowledge areas: epidemiology, molecular biology, genetics, and bioinformatics, and gathered a large genomic dataset to explore similarities in the number of cold tolerance genes in our sequence data collection between human and poultry. In realities where this knowledge and data are not readily available, the usage of NGRA may not be feasible. Still, we were limited by the scarcity of experimental data mapping CTGs into cold inactivation. It is already discussed in the literature that the lack of knowledge on the state of the art about how multidimensional genetic data can be translated into tangible phenotypical characteristics for RA implementation (den Besten et al., 2018) is a bottle neck for NGRAs. It could be achieved by pipelining bioinformatics analysis, such as genome-wide association studies, along with the predictive microbiology experiments and machine learn models that can deal with a large amount of information to explore the relationship between genome and microbial dynamics, such as growth, inactivation, adherence, and food characteristics: pH, available water (aw), and temperature (Guo et al., 2021).

Hence, before the implementation of NGRA we still miss a "next generation predictive microbiology" to underpin the relationships between genetic features and relevant phenotypic characteristics for risk assessment, such as thermal resistance, survival, growth, or virulence. This could substantiate several assumptions we are taking, such as the independence between all CTGs and the shape of the three inactivation curves' effect on the cold inactivation assumption. Currently, the impact

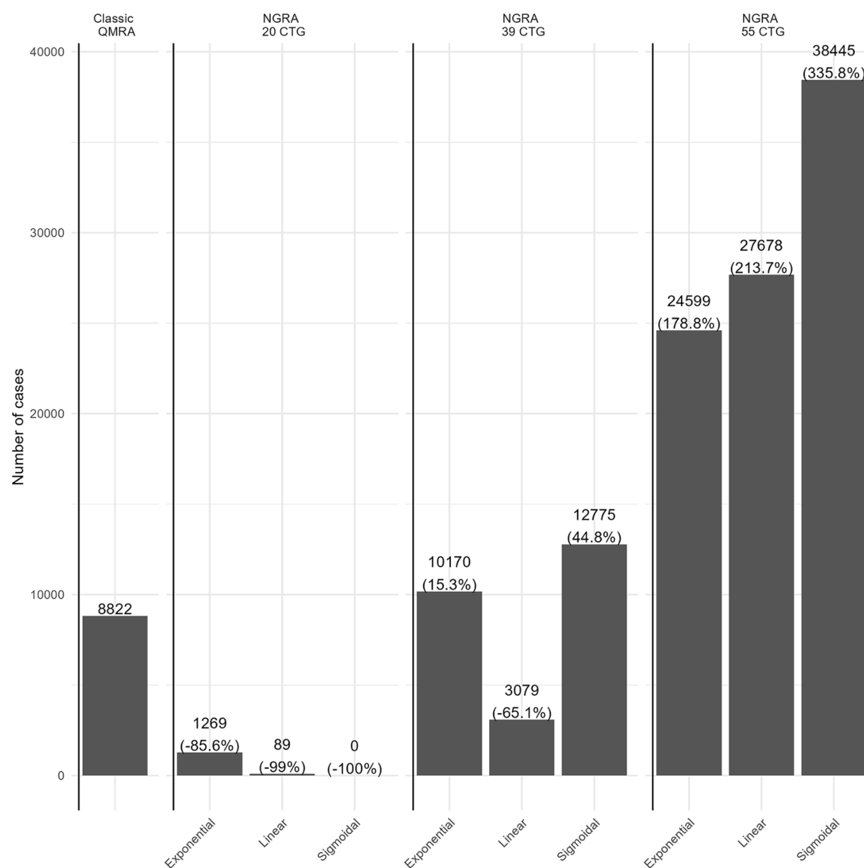

Fig. 5. Expected number of human campylobacteriosis cases caused by the consumption of salad contaminated by cross-contamination from chicken filets during meal preparation, as estimated by the classic QMRA and the NGRA model. Each bar corresponds to the combination of one cold inactivation model (sigmoidal, linear, and exponential) with a NGRA model scenario: 20, 39, and 55 cold tolerance genes (CTGs).

of these assumptions on the results remains uncertain. Here, we could explore that the cold inactivation model (i.e., sigmoidal, linear, and exponential) impacts the risk estimate. Thus, further studies in the field of predictive microbiology should explore the interface of genotype and phenotype, bringing a more detailed mathematical expression for NGRA models.

Campylobacteriosis has been the leading food-borne zoonosis in the EU since 2005 (EFSA/ECDC, 2024), and the results of the CARMA project were extensively used to set up risk mitigation strategies in Europe, such as the microbiological criteria for broiler carcasses contamination (Regulation EC No. 2017/1495, 2017). Building on the top of that, we have chosen to explore the campylobacter survival during storage conditions, due to the available information on identified genes (Hur et al., 2022), the availability of sequences allowing for testing the presence of these genes in different reservoirs (Mughini-Gras et al., 2021), and model feasibility. Despite the observation that neglecting the hazard’s genetic variability may result in over- or underestimation of microbial exposure and associated risk, we still cannot propose a direct use of NGRA in risk mitigation strategies.

Although not exhaustive, other implementation of genomics int QMRA are available for instance in Fritsch et al. (2018) who explored clustering the genes depending on the virulence of L. *monocytogenes*, and concluded that risk management of listeriosis in cold smoked salmon should be targeted to monitor the presence of the specific clonal complexes of bigger virulence. In Godínez-Oviedo et al. (2022), the authors discuss that the ingested dose of salmonella in chicken meat in Mexico was not the best predictor of the probability of illness. The authors conclude that genotypic and phenotypic characteristics of the strains are more relevant and consequently, it should shape new risk assessments. It was also concluded that the risk of listeriosis by the consumption of cultured milk products, is driven by the distribution of number of sub-populations for each of the stress phenotypes (acid, cold, salt, and desiccation) of L. *monocytogenes*; however the authors do not disclaim a clear application of the findings into risk mitigation strategies (Njage et al., 2020). All the mentioned works also point to the fact that there is still a need for translating the multidimensional genetic data into parameters/metrics that match the stakeholders' requirements towards risk management and mitigation (Franz et al., 2016).

Although the integration of genomics into QMRA has been already explored, to our knowledge, this is the first attempt to update *Campylobacter* QMRA with genomic data. We successfully incorporated cold tolerance genes of *Campylobacter* into conventional QMRA, creating the NGRA. We provide proof of concept for incorporating whole-genome sequence data into risk assessment. Our findings show that accounting for genomic variability in gene content can influence risk estimates. This approach could be extended to other foodborne hazards, including *E. coli, Salmonella*, and *Listeria.* We also complement the available scientific literature on the awareness that, currently there is limited knowledge on how genomic data can be translated to phenotypical characteristics and further integrated into QMRA models.

CRediT authorship contribution statement

Eduardo de Freitas Costa: Writing – review & editing, Writing – original draft, Software, Methodology, Funding acquisition, Formal analysis, Conceptualization. **Andries A. Kampfraath:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Funding acquisition, Data curation, Conceptualization. **Dirkjan Schokker:** Writing – review & editing, Writing – original draft, Methodology, Funding acquisition, Conceptualization. **Menno van der Voort:** Writing

– review & editing, Writing – original draft, Conceptualization. **Roan Pijnacker:** Writing – review & editing, Writing – original draft, Conceptualization. **Clazien J. de Vos:** Writing – review & editing, Writing – original draft, Methodology, Funding acquisition, Conceptualization. **Eric G. Evers:** Writing – review & editing, Writing – original draft, Supervision, Methodology, Conceptualization. **Alex Bossers:** Writing – review & editing, Writing – original draft, Methodology, Funding acquisition, Conceptualization. **Jose L. Gonzales:** Writing – review & editing, Writing – original draft, Methodology, Funding acquisition, Conceptualization. **Ewa Pacholewicz:** Writing – review & editing, Writing – original draft, Supervision, Project administration, Methodology, Funding acquisition, Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.mran.2026.100365.

Data availability

gitlab repo with data and scripts

References

Arnaboldi, S., Benincà, E., Evers, E.G., 2023. Improvement of quantitative microbiological risk assessment (QMRA) methodology through integration with gaenetic data. EFSa J. 21. https://doi.org/10.2903/j.efsa.2023.e211003.

Carbo, E.C., Mourik, K., Boers, S.A., Munnink, B.O., Nieuwenhuijse, D., Jonges, M., Welkers, M.R.A., Matamoros, S., van Harinxma thoe Slooten, J., Kraakman, M.E.M., Karelioti, E., van der Meer, D., Veldkamp, K.E., Kroes, A.C.M., Sidorov, I., de Vries, J. J.C., 2023. A comparison of five Illumina, Ion Torrent, and nanopore sequencing technology-based approaches for whole genome sequencing of SARS-CoV-2. Eur. J. Clin. Microbiol. Infect. Dis. 42, 701–713. https://doi.org/10.1007/s10096-023-04590-0.

Cocolin, L., Mataragas, M., Bourdichon, F., Doulgeraki, A., Pilet, M.-F., Jagadeesan, B., Rantsiou, K., Phister, T., 2018. Next generation microbiological risk assessment meta-omics: the next need for integration. Int. J. Food Microbiol. 287, 10–17. https://doi.org/10.1016/j.ijfoodmicro.2017.11.008.

Collineau, L., Boerlin, P., Carson, C.A., Chapman, B., Fazil, A., Hetman, B., McEwen, S.A., Parmley, E.J., Reid-Smith, R.J., Taboada, E.N., Smith, B.A., 2019. Integrating whole-genome sequencing data into quantitative risk assessment of foodborne antimicrobial resistance: a review of opportunities and challenges. Front. Microbiol. 10. https://doi.org/10.3389/fmicb.2019.01107.

Correia Carreira, G., Projahn, M., Langkabel, N., Becker, E., Käsbohrer, A., 2023. Modeling of interventions for reducing external *Enterobacteriaceae* contamination of broiler carcasses during processing. Risk. Anal. 43, 1933–1945. https://doi.org/10.1111/risa.14079.

Damjanova, I., Jakab, M., Farkas, T., Mészáros, J., Galántai, Z., Turcsányi, I., Bistyák, A., Juhász, Á., Pászti, J., Kiss, I., Kardos, G., 2011. From farm to fork follow-up of thermotolerant campylobacters throughout the broiler production chain and in human cases in a Hungarian county during a ten-months period. Int. J. Food Microbiol. 150, 95–102. https://doi.org/10.1016/j.ijfoodmicro.2011.07.011.

den Besten, H.M.W., Amézquita, A., Bover-Cid, S., Dagnas, S., Ellouze, M., Guillou, S., Nychas, G., O'Mahony, C., Pérez-Rodriguez, F., Membré, J.-M., 2018. Next generation of microbiological risk assessment: potential of omics data for exposure assessment. Int. J. Food Microbiol. 287, 18–27. https://doi.org/10.1016/j.ijfoodmicro.2017.10.006.

EFSA/ECDC, 2024. The European Union one Health 2023 zoonoses report. EFSa J. 22. https://doi.org/10.2903/j.efsa.2024.9106.

Franz, E., Gras, L.M., Dallman, T., 2016. Significance of whole genome sequencing for surveillance, source attribution and microbial risk assessment of foodborne pathogens. Curr. Opin. Food Sci. 8, 74–79. https://doi.org/10.1016/j.cofs.2016.04.004.

Fritsch, L., Guillier, L., Augustin, J.-C., 2018. Next generation quantitative microbiological risk assessment: refinement of the cold smoked salmon-related listeriosis risk model by integrating genomic data. Microb. Risk. Anal. 10, 20–27. https://doi.org/10.1016/j.mran.2018.06.003.

Godínez-Oviedo, A., Sampedro, F., Bowman, J.P., Garcés-Vega, F.J., Hernández-Iturriaga, M., 2022. Genotypic and phenotypic quantitative microbial risk assessment model of human salmonellosis related to the consumption of chicken meat in the central region of Mexico. Food Res. Int. 162, 111901. https://doi.org/10.1016/j.foodres.2022.111901.

Guo, X., Song, Y., Liu, S., Gao, M., Qi, Y., Shang, X., 2021. Linking genotype to phenotype in multi-omics data of small sample. BMC Genom. 22, 537. https://doi.org/10.1186/s12864-021-07867-w.

Haddad, N., Johnson, N., Kathariou, S., Métris, A., Phister, T., Pielaat, A., Tassou, C., Wells-Bennik, M.H.J., Zwietering, M.H., 2018. Next generation microbiological risk assessment—Potential of omics data for hazard characterisation. Int. J. Food Microbiol. 287, 28–39. https://doi.org/10.1016/j.ijfoodmicro.2018.04.015.

Hur, J.I., Kim, J., Ryu, S., Jeon, B., 2022. Phylogenetic association and genetic factors in Cold stress tolerance in Campylobacter jejuni. Microbiol. Spectr. 10. https://doi.org/10.1128/spectrum.02681-22.

Koutsoumanis, K., Allende, A., Alvarez-Ordóñez, A., Bolton, D., Bover-Cid, S., Chemaly, M., Davies, R., De Cesare, A., Hilbert, F., Lindqvist, R., Nauta, M., Peixe, L., Ru, G., Simmons, M., Skandamis, P., Suffredini, E., Jenkins, C., Malorny, B., Ribeiro Duarte, A.S., Torpdahl, M., da Silva Felício, M.T., Guerra, B., Rossi, M., Herman, L., 2019. Whole genome sequencing and metagenomics for outbreak investigation, source attribution and risk assessment of food-borne microorganisms. EFSa J. 17. https://doi.org/10.2903/j.efsa.2019.5898.

Lindqvist, R., Sylvén, S., Vågsholm, I., 2002. Quantitative microbial risk assessment exemplified by Staphylococcus aureus in unripened cheese made from raw milk. Int. J. Food Microbiol. 78, 155–170. https://doi.org/10.1016/S0168-1605(02)00237-4.

Mughini-Gras, L., Pijnacker, R., Coipan, C., Mulder, A.C., Fernandes Veludo, A., de Rijk, S., van Hoek, A.H.A.M., Buij, R., Muskens, G., Koene, M., Veldman, K., Duim, B., van der Graaf-van Bloois, L., van der Weijden, C., Kuiling, S., Verbruggen, A., van der Giessen, J., Opsteegh, M., van der Voort, M., Castelijn, G.A. A., Schets, F.M., Blaak, H., Wagenaar, J.A., Zomer, A.L., Franz, E., 2021. Sources and transmission routes of campylobacteriosis: a combined analysis of genome and exposure data. J. Infect. 82, 216–226. https://doi.org/10.1016/j.jinf.2020.09.039.

Mughini-Gras, L., Smid, J.H., Wagenaar, J.A., de Boer, A.G., Havelaar, A.H., Friesema, I. H.M., French, N.P., Busani, L., van Pelt, W., 2012. Risk factors for campylobacteriosis of chicken, ruminant, and environmental origin: a combined case-control and source attribution analysis. PLoS. One 7, e42599. https://doi.org/10.1371/journal.pone.0042599.

Murphy, C., Carroll, C., Jordan, K.N., 2006. Environmental survival mechanisms of the foodborne pathogen Campylobacter jejuni. J. Appl. Microbiol. 100, 623–632. https://doi.org/10.1111/j.1365-2672.2006.02903.x.

Nauta, M., 2005. Microbiological risk assessment models for partitioning and mixing during food handling. Int. J. Food Microbiol. 100, 311–322. https://doi.org/10.1016/j.ijfoodmicro.2004.10.027.

Nauta, M., Hill, A., Rosenquist, H., Brynestad, S., Fetsch, A., van der Logt, P., Fazil, A., Christensen, B., Katsma, E., Borck, B., Havelaar, A., 2009. A comparison of risk assessments on Campylobacter in broiler meat. Int. J. Food Microbiol. 129, 107–123. https://doi.org/10.1016/j.ijfoodmicro.2008.12.001.

Nauta M., Jacobs-Reitsma W. F., Evers E., G van Pelt W., Havelaar A. H., 2005. Risk assessment of Campylobacter in the Netherlands via broiler meat and other routes. Bilthoven.

Nauta, M.J, 2002. Modelling bacterial growth in quantitative microbiological risk assessment: is it possible? Int. J. Food Microbiol. 73, 297–304. https://doi.org/10.1016/S0168-1605(01)00664-X.

Njage, P.M.K., Leekitcharoenphon, P., Hald, T., 2019. Improving hazard characterization in microbial risk assessment using next generation sequencing data and machine learning: predicting clinical outcomes in shigatoxigenic *Escherichia coli*. Int. J. Food Microbiol. 292, 72–82. https://doi.org/10.1016/j.ijfoodmicro.2018.11.016.

Njage, P.M.K., Leekitcharoenphon, P., Hansen, L.T., Hendriksen, R.S., Faes, C., Aerts, M., Hald, T., 2020. Quantitative microbial risk assessment based on whole genome sequencing data: case of Listeria monocytogenes. Microorganisms. 8, 1772. https://doi.org/10.3390/microorganisms8111772.

Pires, S.M., Duarte, A.S., Hald, T., 2018. Source attribution and risk assessment of antimicrobial resistance. Antimicrobial Resistance in Bacteria from Livestock and Companion Animals. ASM Press, Washington, DC, USA, pp. 619–635. https://doi.org/10.1128/9781555819804.ch29.

Pires, S.M., Redondo, H.G., Pessoa, J., Jakobsen, L.S., Thomsen, S.T., 2024. Risk ranking of foodborne diseases in Denmark: reflections on a national burden of disease study. Food Control 158, 110199. https://doi.org/10.1016/j.foodcont.2023.110199.

Rantsiou, K., Kathariou, S., Winkler, A., Skandamis, P., Saint-Cyr, M.J., Rouzeau-Szynalski, K., Amézquita, A., 2018. Next generation microbiological risk assessment: opportunities of whole genome sequencing (WGS) for foodborne pathogen surveillance, source tracking and risk assessment. Int. J. Food Microbiol. 287, 3–9. https://doi.org/10.1016/j.ijfoodmicro.2017.11.007.

Regulation EC No. 2017/1495, 2017. Commission Regulation (EU) 2017/1495 of 23 August 2017 amending regulation (EC) No 2073/2005 as regards Campylobacter in broiler carcases, 1495. Off. J. Eur. Union.

Rosner, B.M., Schielke, A., Didelot, X., Kops, F., Breidenbach, J., Willrich, N., Gölz, G., Alter, T., Stingl, K., Josenhans, C., Suerbaum, S., Stark, K., 2017. A combined case-control and molecular source attribution study of human Campylobacter infections in Germany, 2011–2014. Sci. Rep. 7, 5139. https://doi.org/10.1038/s41598-017-05227-x.

Rutherford, K., Parkhill, J., Crook, J., Horsnell, T., Rice, P., Rajandream, M.-A., Barrell, B., 2000. Artemis: sequence visualization and annotation. Bioinformatics. 16, 944–945. https://doi.org/10.1093/bioinformatics/16.10.944.

Seemann, T., 2025. Abricate [W W W Document]. https://github.com/tseemann/abricate.

R Core Team, 2023. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Available at: https://www.r-project.org/.

Teunis, P., Schijven, J.F., 2018. Generic Guidance to Quantitative Microbial Risk Assessment For Food and Water. Bilthoven. https://doi.org/10.21945/RIVM-2017-0188.

Teunis, P.F.M., Nagelkerke, N.J.D., Haas, C.N., 1999. Dose response models for infectious gastroenteritis. Risk. Anal. 19, 1251–1260. https://doi.org/10.1023/A:1007055316559.

Thystrup, C., Brinch, M.L., Henri, C., Mughini-Gras, L., Franz, E., Wieczorek, K., Gutierrez, M., Prendergast, D.M., Duffy, G., Burgess, C.M., Bolton, D., Alvarez, J., Lopez-Chavarrias, V., Rosendal, T., Clemente, L., Amaro, A., Zomer, A.L., Grimstrup Joensen, K., Nielsen, E.M., Scavia, G., Skarżyńska, M., Pinto, M., Oleastro, M., Cha, W., Thépault, A., Rivoal, K., Denis, M., Chemaly, M., Hald, T., 2025. Source attribution of human Campylobacter infection: a multi-country model in the European Union. Front. Microbiol. 16. https://doi.org/10.3389/fmicb.2025.1519189.

Vidal, A.B., Colles, F.M., Rodgers, J.D., McCarthy, N.D., Davies, R.H., Maiden, M.C.J., Clifton-Hadley, F.A., 2016. Genetic diversity of Campylobacter jejuni and Campylobacter coli isolates from conventional broiler flocks and the impacts of sampling strategy and laboratory method. Appl. Environ. Microbiol. 82, 2347–2355. https://doi.org/10.1128/AEM.03693-15.

Wetterstrand, K.A., 2023. DNA Sequencing Costs: Data from the NHGRI Genome Sequencing Program (GSP) [W W W Document]. URL. https://www.genome.gov/about-genomics/fact-sheets/DNA-Sequencing-Costs-Data (accessed 9.19.25).

WHO, 2021. Microbiological Risk Assessment – Guidelines for Food. FAO and WHO, Rome. https://doi.org/10.4060/cb5006en.