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PHD IN FOOD SCIENCE
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Microbiome Mapping in Meat Food Chain from Farm-to-Fork

Tutor:

Prof. Francesca De Filippis

PhD Candidate:

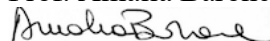
Asim ur Rahman

Co-Tutor:

Prof. Avelino Álvarez Ordóñez

Coordinator:

Prof. Amalia Barone



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RIASSUNTO CAPITOLO 1

Questo primo capitolo della mia tesi è una revisione meta-analitica su larga scala, che esplora il diffuso e sistematico problema della resistenza antimicrobica (AMR) nel nostro ambiente, di cui l'allevamento animale è considerato uno dei principali motori. Pertanto, l'obiettivo principale era confrontare gli allevamenti convenzionali (che usano regolarmente antibiotici) con gli allevamenti privi di antibiotici (ABF) per indagare le differenze nella presenza qualitativa e quantitativa dei geni di resistenza antimicrobica (ARG).

Dopo aver raccolto e combinato statisticamente i dati di 37 studi pubblicati negli ultimi dieci anni (2014-2024), i risultati hanno mostrato due scoperte chiave. In primo luogo, come previsto, gli allevamenti convenzionali avevano una probabilità più che doppia di ospitare ARG, a causa dell'uso regolare di antimicrobici.

Tuttavia, la scoperta più critica e sorprendente è stata che il 97% dei cosiddetti allevamenti “privi di antibiotici” ospitava ancora geni di resistenza. Ciò dimostra che la sola interruzione dell'uso di antibiotici, sebbene importante, non è una soluzione completa. Il problema è molto più complesso. I geni di resistenza persistono chiaramente nell'ambiente dell'allevamento, nel suolo e nei reflui animali anche senza la pressione diretta degli antibiotici. Questo è probabilmente dovuto al fatto che l'AMR è un problema più ampio, guidato da molti fattori, tra cui l'uso di antimicrobici nella medicina umana, lo smaltimento improprio di disinfettanti domestici e il commercio globale, che contribuiscono tutti all'emergenza e alla disseminazione della resistenza nell'ambiente.

La conclusione principale di questo capitolo è che la resistenza agli antibiotici è un problema globale di “One Health”. Non è solo un problema di allevamento animale; è una questione condivisa che collega agricoltura, salute umana e ambiente, e deve essere affrontata da tutte le angolazioni.

RIASSUNTO CAPITOLO 2

Il secondo capitolo della tesi è una rassegna scientifica (review) che ha affrontato l'enorme problema globale del deterioramento della carne, che spreca miliardi di dollari e quantità massicce di cibo. La rassegna si è concentrata su tutte le diverse cause del deterioramento della carne (ad esempio, fisiche, chimiche e biologiche), con un'enfasi speciale sui fattori biologici del deterioramento della carne. Poi abbiamo confrontato i pro e i contro di tutti i diversi metodi

che abbiamo sviluppato negli ultimi 20 anni per identificare e tracciare i microbi responsabili di questo deterioramento.

La rassegna ha esaminato tutto, dalla coltura di laboratorio "vecchia scuola" ai moderni approcci high-tech basati sul DNA e "multi-omici", fino ai nuovi biosensori intelligenti. Il metodo tradizionale di cercare di far crescere i batteri in laboratorio è spesso superato perché non riesce a rilevare molti dei microbi ambientali che sono difficili da coltivare. Al contrario, la moderna metagenomica shotgun (che sequenzia tutto il DNA in un campione) è incredibilmente potente. Fornisce un quadro completo e imparziale di tutti i batteri presenti (sia in quantità che in tipo) e, soprattutto, di ciò che i loro geni permettono loro di fare (il loro "potenziale funzionale").

Tuttavia, abbiamo riscontrato una grave lacuna: questi potenti strumenti basati sul DNA sono per lo più confinati nei laboratori di ricerca. Sono generalmente troppo lenti, costosi e complicati per l'uso quotidiano in un'industria della carne dai ritmi serrati. D'altra parte, stanno emergendo nuovi strumenti come i "sensori intelligenti" o i "nasi elettronici", che sono veloci e possono essere utilizzati direttamente sulla linea di produzione, ma non forniscono i dati ricchi e dettagliati che offre il sequenziamento.

La conclusione principale di questo capitolo è che il futuro della sicurezza alimentare non è una scelta "aut aut". La soluzione migliore sarà un sistema ibrido che combini la velocità dell'IA e dei sensori intelligenti con il dettaglio genetico profondo della metagenomica. Affinché ciò diventi realtà, la rassegna ha identificato tre necessità urgenti: 1) dobbiamo standardizzare i nostri metodi, in modo che i risultati siano comparabili, 2) dobbiamo costruire database migliori di geni correlati al deterioramento, e 3) abbiamo bisogno di supporto politico per aiutare le aziende a permettersi e adottare queste nuove tecnologie.

RIASSUNTO CAPITOLO 3

Il terzo capitolo è stato lo studio sperimentale principale del mio dottorato, in cui abbiamo condotto un'indagine su larga scala per mappare l'intero mondo microbico dell'industria bovina. Utilizzando il sequenziamento avanzato del DNA, questo lavoro ha tracciato i microbi di quattro bovini partendo dalla loro fattoria, attraverso quattro diversi impianti di lavorazione, fino ai prodotti finiti di carne stagionata (sia porzioni a lunga stagionatura che a livello di vendita al dettaglio). L'intera strategia di campionamento è stata ripetuta sia in estate che in

inverno per indagare l'impatto delle stagioni sulle dinamiche del microbioma. Abbiamo anche confrontato le superfici durante la produzione attiva rispetto a subito dopo la pulizia per comprendere gli effetti della sanificazione.

I risultati hanno mostrato che un impianto di lavorazione della carne è un ecosistema vivente con le sue complesse regole ecologiche. Abbiamo riscontrato una sorprendente "inversione stagionale": in estate, l'ambiente dell'impianto stesso era più ricco di diversità microbica e dei geni di rischio associati (ad esempio, geni di deterioramento, biocidi, virulenza e resistenza antimicrobica). In inverno, i prodotti finali di carne stagionata mostravano il contrario, finendo per avere una maggiore diversità microbica, inclusa una quantità elevata e diversificata di geni di resistenza agli antibiotici.

Una scoperta fondamentale è stata che la sanificazione di routine non si limita a resettare il sistema. Agisce come un potente filtro. Sebbene la pulizia rimuovesse alcuni batteri responsabili del deterioramento, permetteva anche ai microbi più resistenti e resilienti (specialmente quelli che formano "slime" protettivi, o biofilm) di sopravvivere. Ciò ha creato un paradosso: le superfici dopo la pulizia erano arricchite di geni problematici, mostrando un aumento fino a 12 volte dei geni correlati alla resistenza ai biocidi e alla formazione di biofilm.

Il processo di maturazione stesso ha rivelato anche una battaglia ecologica chiave. La lunga stagionatura indisturbata ha favorito un tipo di genere batterico (*Carnobacterium*), mentre la "stagionatura al dettaglio" più breve, che inizia dopo il taglio della carne, ha favorito un tipo diverso a crescita rapida (*Lactococcus*). Infine, lo studio ha mostrato che i virus che uccidono i batteri (batteriofagi) svolgono un ruolo nascosto. Sebbene per lo più sembrano coesistere pacificamente con i batteri, abbiamo trovato situazioni specifiche in cui stavano attivamente cacciando e sopprimendo patogeni chiave come *Listeria* e *Salmonella*, agendo come una forma naturale di controllo della sicurezza.

Nel complesso, questo capitolo ha mostrato che la sicurezza e il deterioramento della carne non sono governati da un singolo fattore. Sono il risultato di una complessa competizione ecologica, modellata dalla stagione, dagli effetti reali della pulizia e dalla predazione nascosta tra batteri e i loro rispettivi batteriofagi

SUMMARY CHAPTER 1

This first chapter of my thesis is a large-scale meta-analysis review, exploring the widespread and systematic problem of antimicrobial resistance (AMR) in our environment, for which animal farming is considered one of the leading drivers. Therefore, the main goal was to compare conventional farms (which regularly use antibiotics) with antibiotic-free farms (ABF) to investigate the differences in the qualitative and quantitative presence of antimicrobial resistance genes (ARGs).

After gathering and statistically combining the data from 37 studies published in the last ten years (2014-2024), the results showed two key findings. First, as expected, the conventional farms were more than twice as likely to have ARGs, because of the regular use of antimicrobials.

However, the most critical and surprising finding was that 97% of the so-called “antibiotic-free” farms still harboured resistance genes. This shows that just stopping antibiotic use, while important, is not a complete solution. The problem is much more complex. The resistance genes are clearly persisting in the farm environment, in the soil and in animal waste even without the direct pressure of antibiotics. This is likely because AMR is a wider issue, driven by many factors, including antimicrobial use in human medicine, improper disposal of household disinfectants, and global trade, all of which contribute to the emergence and dissemination of resistance in the environment.

The main conclusion from this chapter is that antibiotic resistance is a global “One Health” problem. It's not just an animal farming problem; it's a shared issue that connects agriculture, human health, and the environment, and it needs to be tackled from all angles.

SUMMARY CHAPTER 2

The second thesis chapter is a review paper that tackled the huge global problem of meat spoilage, which wastes billions of dollars and massive amounts of food. The review focused on all the different causes of meat spoilage (e.g., physical, chemical, and biological), with a special emphasis on the biological drivers of meat spoilage. Then we compared the pros and cons of all different methods we've developed over the past 20 years to identify and track the microbes responsible for this spoilage.

SUMMARY (ENGLISH)

The review looked at everything from old-school lab culturing to modern, high-tech DNA and “multi-omics” approaches, and even new smart biosensors. The traditional method of trying to grow bacteria in a lab is often outdated because it fails to detect many of the environmental microbes that are hard to grow. In contrast, modern shotgun metagenomics (which sequences all DNA in a sample) is incredibly powerful. It gives a complete, unbiased picture of all the bacteria present (both quantity and type) and, crucially, what their genes allow them to do (their "functional potential").

However, we found a major gap: these powerful DNA tools are mostly stuck in research labs. They are generally too slow, expensive, and complicated for everyday use in a fast-paced meat industry. On the other hand, new tools like "smart sensors" or "electronic noses" are fast and can be used right on the production line, but they don't provide the rich, detailed data that sequencing does.

The main conclusion from this chapter is that the future of food safety isn't an "either/or" choice. The best solution will be a hybrid system that combines the speed of AI and smart sensors with the deep genetic detail of metagenomics. For this to become a reality, the review identified three urgent needs: 1) we must standardize our methods, so results are comparable, 2) we need to build better databases of spoilage-related genes, and 3) we need policy support to help companies afford and adopt these new technologies.

SUMMARY CHAPTER 3

The third chapter is the main experimental study of my PhD, where we conducted a large-scale investigation to map the complete microbial world of the beef industry. Using advanced DNA sequencing, this work tracked microbes from four cattle's all the way from their farm, through four different processing plants, and onto the final aged meat products (both long-term aged and retail-stage portions). This entire sampling strategy was repeated in both summer and winter to investigate the impact the seasons on microbiome dynamics. We also compared surfaces during active production versus right after cleaning to understand the effects of sanitation.

The findings showed that a meat plant is a living ecosystem with its own ecological complex rules. We found a striking “seasonal inversion”: In summer, the factory environment itself was richer in microbial diversity and their associated risk genes (e.g., spoilage, biocides, virulence,

and Antimicrobial Resistance Genes). In winter, the final aged meat products showed the opposite, ending up with more microbial diversity, including a high and diverse amount of antibiotic resistance genes.

A critical discovery was that routine sanitation doesn't just reset the system. It acts as a powerful filter. While cleaning did remove some spoilage bacteria, it also allowed the tougher, more resilient microbes (especially those that form protective slimes, or biofilms) to survive. This created a paradox: the surfaces after cleaning were enriched with problematic genes, showing up to a 12-fold increase in genes related to biocide resistance and biofilm-formation.

The maturation process itself also revealed a key ecological battle. Long, undisturbed aging favoured one type of bacterial genera (*Carnobacterium*), while the shorter “retail” aging that starts after the meat is cut favoured a different, faster-growing type (*Lactococcus*). Finally, the study showed that bacteria-killing viruses (bacteriophages) play a hidden role. While they mostly seem to coexist peacefully with bacteria, we found specific situations where they were actively hunting and suppressing key pathogens like *Listeria* and *Salmonella*, acting as a natural form of safety control.

Overall, this chapter showed that meat safety and spoilage aren't ruled by just one factor. They are the result of a complex ecological competition, shaped by the season, the true effects of cleaning, and the hidden predation between bacteria and their respective bacteriophages.

Overall Aim:

To investigate the microbiome dynamics along meat processing chains from farm-to-fork, using a shotgun metagenomics approach, across 2 different seasons and with a focus on antimicrobial resistance, virulence factors, and bacteriophages influence.

Specific Objectives;

1. To evaluate the effect of seasonality (summer vs winter) on bacterial community structure and functions along different meat processing stages and seasons using shotgun metagenomic sequencing.
2. To identify and quantify key meat relevant spoilers and pathogens along the processing chains.
3. To profile spoilage-related genes, virulence factors, antimicrobial resistance, and biocides resistance genes across processing stages, seasons, and surface types.
4. To identify bacteriophages present in meat processing environments and examine their impact on the abundance and suppression of their predicted bacterial hosts.
5. To assess the effectiveness of routine cleaning and sanitation practices in reducing microbial load, spoilage organisms, resistance genes, and virulence factors.

CHAPTER 1

Comparative Analysis of Antibiotic-Administered vs. Antibiotic-Free Farming in Meat Production: Implications for Health, Environment, and Antibiotic Resistance

Highlights

- ARGs persist in both conventional and antibiotic-free farms.
- Conventional farms showed higher average ARG detections.
- ARGs were reported in 97% of antibiotic-free studies.
- Cattle and farm samples had the highest ARG odds ratios.
- Farm-level interventions are key to controlling ARG spread.

Abstract

Antimicrobial resistance (AMR) is an escalating global health problem, endangering human, animal, and environmental health. In animal farming, the widespread use of antimicrobials is recognized one of the major drivers of AMR. Therefore, this review provides a meta-analysis of 37 studies published between 2014 and 2024, comparing the prevalence of antimicrobial resistance genes (ARGs) in antibiotic-free (ABF) versus conventional animal farming (CONV) systems. The statistical analysis revealed that CONV farms exhibited a higher likelihood of harbouring ARGs, with a pooled odds ratio of 2.38 (95% CI: 2.00–2.83) in the fixed-effects model and 3.21 (95% CI: 1.68–6.13) in the random-effects model. Significant heterogeneity was observed ($I^2 = 82.8\%$, $p < 0.0001$), highlighting the variations across the study designs. However, ARGs were still detected in 97% of ABF farms, suggesting that antibiotic reduction alone may not be enough to control AMR in animal farming. These findings underscore the complex nature of AMR, influenced by environmental contamination, microbial interactions, human practices, and ecological pressures such as climate change. Future strategies should adopt a holistic One Health approach to effectively mitigate AMR risks across sectors and safeguard public and planetary health.

Keywords: *Global AMR, Food Safety, One Health, Environmental Contamination, AMR Transmission, Microbial Evolution, Resistance Genes*

1. Introduction

Antimicrobial resistance (AMR) is currently one of the pressing global health challenges. It limits the effective treatment of infections and increases the risk of disease spread, severe illness, and death. AMR arises when microorganisms, such as bacteria, viruses, and fungi, develop the ability to resist antimicrobial agents, making standard treatments ineffective (Van Boeckel et al., 2019; WHO, 2023). In 2019, bacterial AMR alone was responsible for an estimated 1.27 million deaths worldwide (Murray et al., 2022; WHO, 2023), and projections suggest that, if left unaddressed, AMR could surpass cancer as a leading cause of mortality (O'Neill, 2016).

In animal farming, antimicrobials have long been used not only to treat infections but also as growth promoters, to increase productivity. These practices are particularly common in conventional (CONV) intensive farming systems. However, the overuse and misuse of antibiotics in livestock contribute significantly to the emergence and spread of antibiotic-resistant bacteria (ARB) (Manyi-Loh et al., 2018; Szoke et al., 2025). These bacteria, along with their ARGs, can be transmitted to humans through direct contact, environmental contamination, or consumption of animal products, thus posing a public health risk (Manyi-Loh et al., 2018; Temple and Manteca, 2020).

To mitigate AMR risks in intense animal-based food production systems, alternative farming methods have been developed. The goal of alternative farming practices like organic (OR) and antibiotic-free (ABF) farming is to reduce the selective pressure that leads to resistance by using minimal or no antibiotics. However, current findings remain inconsistent. Some studies report significant differences in resistance levels among farming practices (Guo et al., 2018; Huizinga et al., 2019; Vieira et al., 2023; Buthasane et al., 2023), while others find no significant difference (De Cesare et al., 2022; Doster et al., 2018; Gerzova et al., 2015; Ishengoma et al., 2024). However, a few even indicate higher levels of AMR in ABF farms (Vikram et al., 2017; Li et al., 2020; Schmidt et al., 2021; Alvarado et al., 2022; Li et al., 2022). These discrepancies underscore the need for a comprehensive analysis to clarify the relationship between farming practices and AMR development in food-producing animals.

This study presents a combined systematic review and meta-analysis to evaluate the prevalence of AMR in CONV versus ABF farming systems. By integrating both quantitative and qualitative evidence, we aim to assess the impact of antibiotic use in animal agriculture on resistance spread. In addition, we explore broader environmental reservoirs, community-level

interactions, and farm management factors to offer a more comprehensive overview of AMR persistence. This study uniquely combines meta-analysis and systematic review approaches to address key knowledge gaps, linking statistical findings with a broader AMR context under the One Health framework.

2. Methodology

2.1. Literature Search:

A comprehensive literature search was conducted using Google Scholar, PubMed, and Scopus to identify peer-reviewed, original research articles published in English between 2014 and 2024. A wide range of keywords was employed, retrieving 299 studies. After thorough screening, 88 studies satisfied the selection criteria for inclusion in both the meta-analysis (37 studies) and the systematic review (51 studies). This review was conducted in accordance with PRISMA guidelines (**Figure 1**).

The 37 studies selected for meta-analysis were categorized as follows: Conventional farming (CONV) refers to intensive animal production systems with unrestricted antimicrobial use. Antibiotic-free farming (ABF) describes systems that eliminate antimicrobial use, while organic systems (OR) are characterized by outdoor access for animals and prohibition of antimicrobials (“QIMA/WQS,” n.d.; Brasil, 2009; Vieira et al., 2023). For statistical analysis, ABF and OR were grouped together and compared against the CONV group.

Inclusion Criteria (for Meta-Analysis):

1. Study Type & Timeframe: Original research articles, published in English between 2014 and 2024.
2. Analytical Methods: Studies employing molecular techniques such as PCR, qPCR, or sequencing-based approaches (e.g., amplicon sequencing, shotgun metagenomics) for microbiome or antibiotic resistance gene (ARG) analysis.
3. Comparative Focus: Comparative studies analysing CONV and ABF systems across different animal types and sample origins.
4. Data Scope: Articles reporting on the full microbiome, multiple serotypes, or resistome data (prevalence).

Exclusion Criteria:

- Reviews, meta-analyses, opinion articles, and grey literature (conference abstracts, thesis, reports, blog posts).
- Studies lacking direct comparative data between CONV and ABF.

2.2. Statistical Analysis

All statistical analyses were performed in R (version 4.3.3) using the tidyverse and meta (v5.7-1) packages. First, descriptive statistics were used to explore the proportions of studies reporting ARG prevalence across farming methods (CONV vs ABF), host types (cattle, poultry, and swine), and sample origins (farm & environment, farm & retail product, and retail product only). After noticing high diversity in the targeted ARGs among studies, we tested data normality using the Shapiro–Wilk test. Based on these results, Wilcoxon rank-sum tests were used to compare ARG detections between groups.

For the meta-analysis, we estimated pooled odds ratios (ORs) with 95% confidence intervals (CIs). Both fixed-effects models (Mantel–Haenszel method), assuming a common true effect across studies, and random-effects models (Inverse-Variance method), accounting for variation between studies, were applied. To evaluate inter-study heterogeneity, three approaches were used: Tau-squared (τ^2) was estimated using the Q-profile method. Restricted maximum likelihood was used for more accurate estimation of τ , τ^2 , and their confidence intervals. Cochran’s Q-test was applied to assess the overall heterogeneity among studies. A continuity correction of 0.5 was added to studies with zero events in one or both groups. To better understand potential variations, subgroup analyses were pre-specified by host type and sample origin. Differences between subgroups were tested using the Chi-squared (χ^2) test.

2.3. Limitations:

A major limitation was the diversity in study designs and objectives across retrieved articles. Comparative analyses were complicated by variability in host species, sample locations, sampling periods, antimicrobial administration modalities, and focus (individual ARGs versus full resistomes). Similarly, additional factors like climate, farm size, and location were inconsistently reported. Such variability increases between-study variance (I^2) and may bias pooled estimates. We therefore interpret our findings as indicative of overall trends rather than precise quantitative differences and highlight the need for standardized studies. To address these concerns and to acquire a general overview, we focused on direct comparative results

reporting variations in microbial species and ARG prevalence between farming systems, while broader environmental influences are discussed in (One Health Narrative) section as potential contributing factors to AMR persistence. This systematic approach allowed us to obtain significant insights into the complex interconnected factors influencing AMR within and beyond animal food systems.

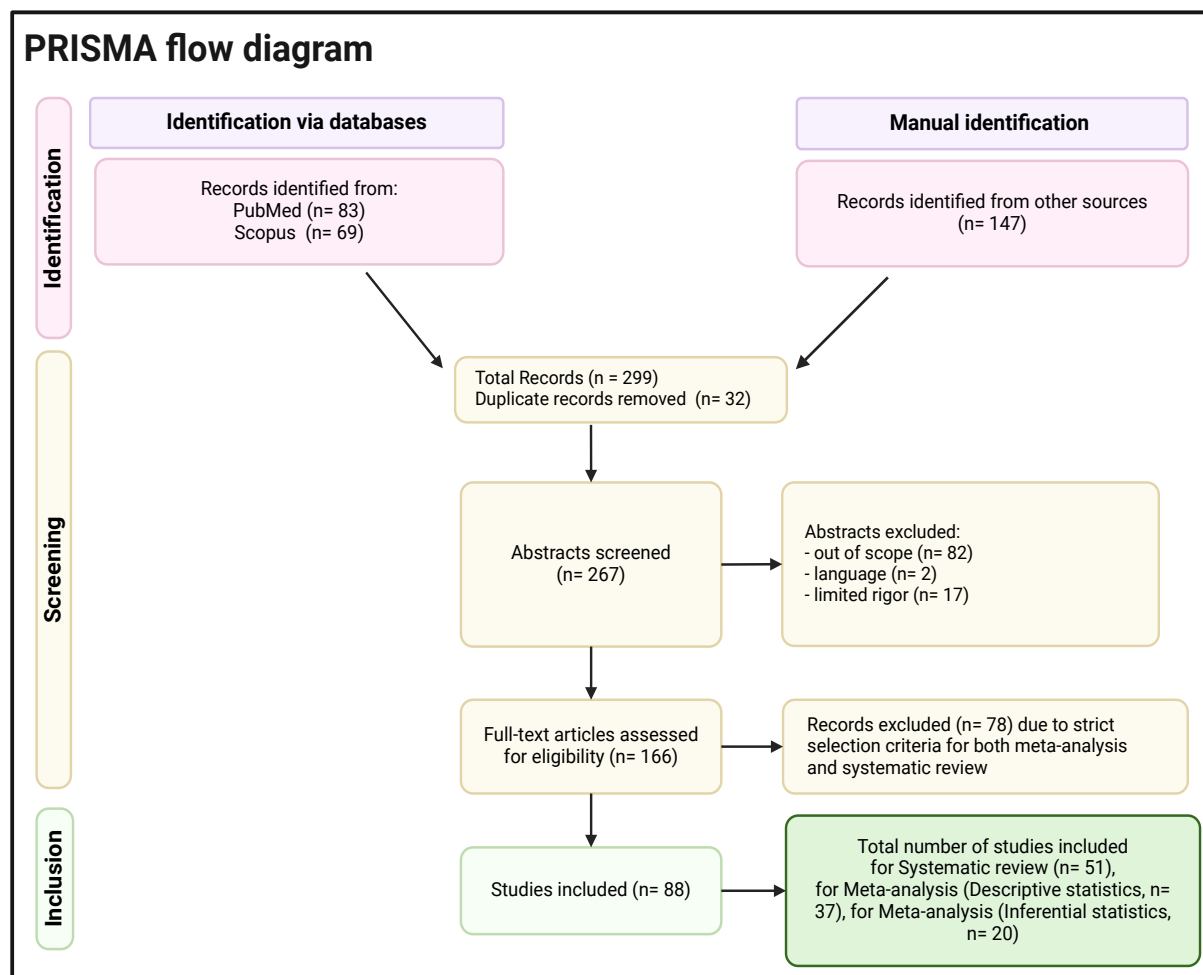


Figure 1. This diagram outlines the systematic process used to identify and filter studies for the integrated meta-analysis and systematic review. A total of 299 records were initially identified from PubMed (n = 83) and Scopus (n = 69). An additional 147 records were identified through manual searches and other sources. With 32 duplicates removed, yielding 267 abstracts for screening. Of these, 166 full-text articles were assessed for eligibility. Based on strict inclusion criteria regarding timeframe, scope, language, and methodological rigor, 78 records were excluded. Ultimately, 37 studies were included in the meta-analysis, and 51 studies were included in the systematic review, resulting in 88 studies overall. *Created in BioRender. RAHMAN, A. (2025) <https://BioRender.com/s43w442>.*

3. Results and Discussion

This study analysed the impact of farming practices (CONV vs ABF) on the composition of microbial species and ARGs prevalence across 37 eligible studies. Among these, 15 studies focused on poultry, 9 on swine, and 13 on cattle. Regarding sample origins, 24 studies examined farm and environmental samples (including faeces, cecum, ileum, litter, feed, and water), 5 included both farm and retail products (such as colon contents, faeces, and carcasses, meat trimmings), and 8 focused on retail products alone (such as chicken breast, minced beef, or retail carcasses). The total number of samples investigated across all studies was 61315 (minimum = 20, mean = 1657, median = 128, maximum = 46937).

Out of the 37 studies, only 3 (8%) reported differences in the prevalence of major microbial phyla, including Firmicutes, Proteobacteria, Actinobacteria, Bacteroidetes, Spirochaetes, and Tenericutes. Regarding microbial species abundance, 20 studies (54%) found no significant difference between CONV and ABF systems, 12 studies (32%) reported higher abundance in CONV, while 5 studies (13%) reported higher abundance in ABF. These findings suggest that farming practices may not strongly influence overall microbial diversity. However, the abundance of specific microbial taxa could still vary depending on farming conditions. Similarly, different studies applied varied detection methods such as shotgun metagenomics, 16S rRNA amplicon sequencing, and qPCR, each measuring relative rather than absolute abundance. Thus, the patterns reported mainly reflect general trends rather than focusing on individual bacterial taxa among the studies.

Regarding ARG prevalence, the data were as follows: total ARGs detected = 2,273; ARGs detected in CONV = 993; ARGs detected in ABF = 764. On average, CONV farms reported 27 ARG detections per study, compared to 21 in ABF systems, although CONV farms showed a slightly higher number of ARG detections. Nevertheless, the statistical difference in prevalence was not significant ($p > 0.05$), either across host types (**Figure 2A**) or sample origins (**Figure 2B**). This suggests that both CONV and ABF practices do not appear to contribute differently to ARG dissemination in animal farming. Despite reduced or eliminated antibiotic use in ABF systems, factors such as cross-contamination, farm management practices, and environmental reservoirs (like water, air, and feed) likely maintain the spread of ARGs. This pattern may also reflect the widespread use of similar antimicrobial treatments across conventional cattle, swine, and poultry operations, for example, all three often receive the same antibiotic classes (e.g., tetracyclines, β -lactams, macrolides).

Regarding the composition of ARG classes among the 37 studies included in our meta-analysis, ARGs conferring tetracycline resistance were detected in 96% of studies, followed by ARGs associated with multiple antibiotic classes in 80% of studies, aminoglycoside-resistance genes in 54%, beta-lactams in 50%, and macrolide-lincosamide-streptogramin (MLS) genes in 18% of studies, respectively. All species showed tetracycline resistance as dominant, followed by multiple ARGs. Across host types, cattle and poultry showed high diversity in ARG classes as compared to swine, but with varied abundance (**Figure 3A**). When stratified by sample origin, cattle showed high diversity as compared to poultry and swine in the farm and environmental samples. In farm samples and retail product samples, only multiple ARGs were detected; in contrast, poultry products from retail samples exhibited higher ARG diversity than beef products. These results also indicate, that while tetracycline resistance predominates all other classes across all farming systems, cattle farming may contribute more to ARG diversity compared to poultry and swine (**Figure 3B**). However, in farm and retail product samples, poultry displayed a relatively balanced distribution between tetracycline, beta-lactams, and multiple ARGs. Although fewer studies investigated ARGs in retail meat, available evidence suggests that farming practices may have limited influence on resistomes at the retail stage. For example, De Cesare et al. (2022) reported no significant difference in ARG abundance between retail products coming from different farming systems, underscoring the need for further research (De Cesare et al., 2022). Furthermore, when examining ARG abundance, 15 studies reported higher ARG levels in CONV systems compared to 5 studies favouring ABF, while 17 studies found no significant differences.

To further examine the number of ARGs detected across farming systems, we performed a meta-analysis using the meta package in R. In the initial descriptive analysis, 17 studies showed no significant difference in ARG prevalence between CONV and ABF systems. Therefore, to reduce the influence of these null effects and to better estimate the true impact of antibiotic use on ARG prevalence, we excluded these 17 studies from the primary meta-analysis. This step helped minimize bias and allowed a clearer comparison between CONV and ABF farming practices. However, to ensure transparency and address any concerns about this study selection, forest plots including all 37 studies (including the 17 null-effect reports) covering both host types and sample origins are provided in the supplementary materials (**Supplementary Figures S1 and S2**).

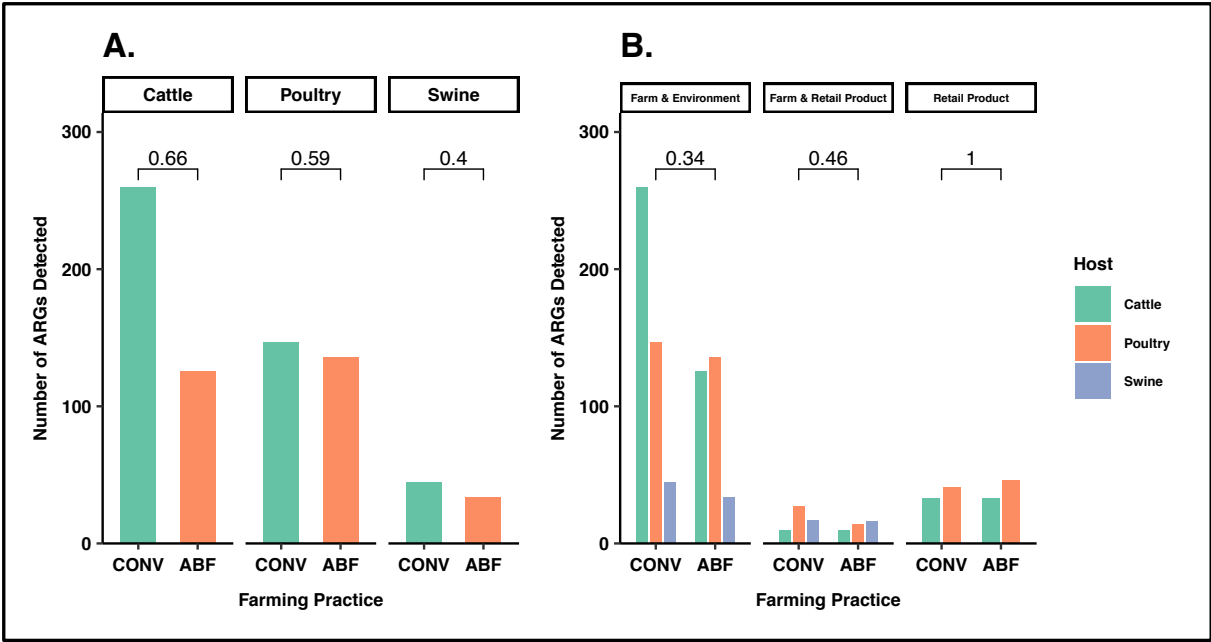


Figure 2. (A) Number of detected ARGs across different host types. Cattle exhibited the highest number of ARG detections, followed by poultry and swine. However, Wilcoxon rank-sum tests showed no statistically significant differences between the groups. Values above the brackets are P-values from pairwise Wilcoxon rank-sum tests (all $p > 0.05$). **(B)** ARG counts further classified by both host type and sample origin. In farm and environmental samples, cattle had the highest number of detected ARGs, followed by poultry. In contrast, poultry showed higher ARG detection in retail product samples compared to cattle.

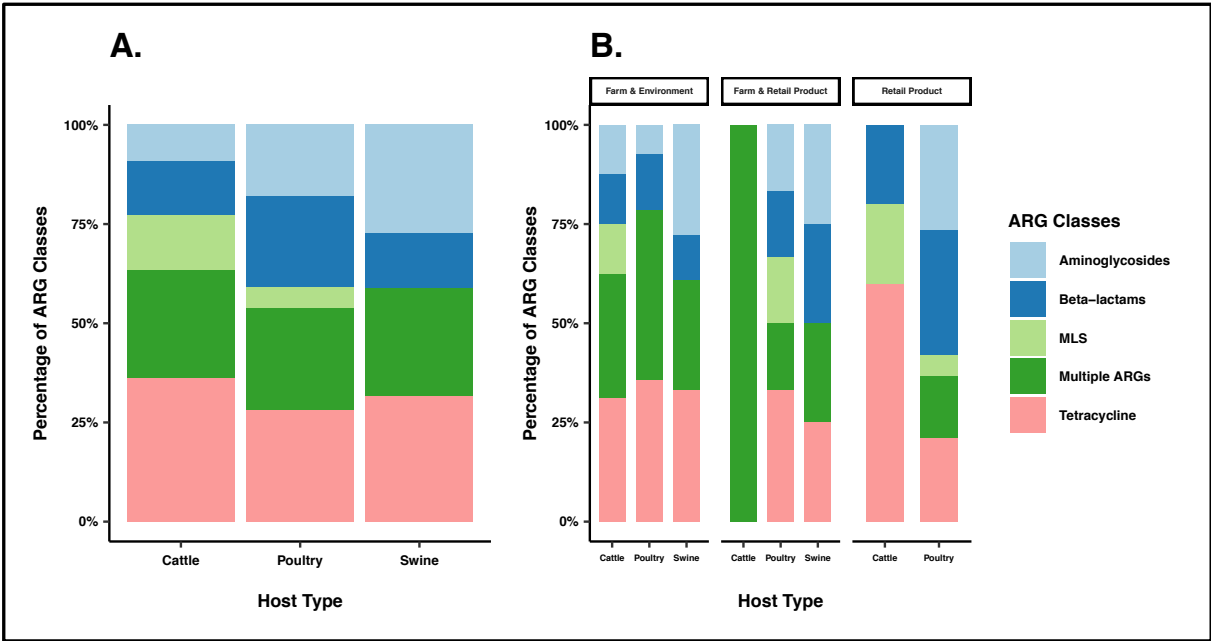


Figure 3. (A) Distribution of ARG classes among host types. While cattle and poultry exhibited similar ARG class diversity, their relative prevalence varied. Swine samples showed lower ARG class diversity compared to both cattle and poultry. **(B)** ARG class distribution further stratified by host type and sample origin. In farm and environmental samples, cattle had the highest ARG class diversity, while poultry showed higher diversity in both farm and retail product samples, as well as retail-only samples.

3.1. Subgroup Analysis by Host Type

In total, 2570 observations from 20 studies were analyzed, with an equal number of samples from CONV ($n = 1285$) and ABF ($n = 1285$) farming systems. Across these, 1339 ARG detection events were recorded. The fixed-effects model showed that animals raised in CONV systems were significantly more likely to carry ARGs, with an odds ratio (OR) of 2.38 (95% CI: 2.00–2.83; $p < 0.0001$). Using the random-effects model, the association was even stronger, with an OR of 3.21 (95% CI: 1.68–6.13; $p = 0.0004$). The random-effects model was chosen to account for differences between studies, like sample types, study designs, and animal host types.

There was significant heterogeneity found across studies ($I^2 = 82.8\%$, $\tau^2 = 1.4809$, $\tau = 1.2169$). Further confirmed by Q test ($Q = 110.61$; $p < 0.0001$). We found very high between-study heterogeneity ($I^2 = 82.8\%$; $Q = 110.6$, $p < 0.0001$), indicating that our pooled ORs describe broad trends rather than exact magnitudes. This variability likely arises not only from study design differences (targeted ARGs vs. full resistomes) but also from unmeasured factors such as farm scale, regional climate, biosecurity practices, feed/water quality, and detection methods, that our meta-analysis cannot disentangle.

When grouped by host type under a fixed-effects model, swine exhibited the highest odds of ARG detection (OR = 3.49; 95% CI: 1.96–6.20; $I^2 = 37.6\%$), followed by cattle (OR = 3.30; 95% CI: 2.58–4.21; $I^2 = 73.7\%$) and poultry (OR = 1.38; 95% CI: 1.04–1.84; $I^2 = 86.5\%$). The subgroup test showed significant differences among species ($Q = 22.63$; $p < 0.0001$) (**Figure 4**). However, under the random-effects model, the differences between cattle, poultry, and swine were not significant ($Q = 0.17$; $p = 0.9199$). Again, the high I^2 values in some subgroups (e.g. 86.5% for poultry) underscore that these species-level comparisons reflect overarching patterns more than definitive quantitative differences. This suggests that even though cattle and swine may have higher ARG levels in some studies, the overall trend is similar when study variability is taken into account.

3.2. Subgroup Analysis by Sample Origin

A similar pattern was seen when we divided the data by where samples were collected (sample location). Both fixed and random-effects models showed that CONV farms had a significantly higher risk of ARG detection compared to ABF farms. Samples collected from farm and environment showed the highest odds for ARG detection (Fixed-effect OR = 3.84; 95% CI: 3.08–4.79; $I^2 = 68.8\%$). Farm and retail product samples had an OR of 2.62 (95% CI: 1.39–4.97; $I^2 = 0.0\%$). However, retail product samples had even a lower OR of 0.65 (95% CI: 0.45–0.94; $I^2 = 85.7\%$), suggesting a possible reduction in ARGs after processing.

The subgroup differences were significant ($Q = 65.99$; $p < 0.0001$), confirming that where the sample came from matters. In the random-effects model, the trend stayed the same: farm samples had the highest OR (7.31; 95% CI: 3.12–17.13), followed by farm and retail samples (2.63; 95% CI: 1.38–5.01). Retail products again showed a low OR (0.83; 95% CI: 0.27–2.57), with significant subgroup differences ($Q = 9.34$; $p = 0.0094$) (**Figure 5**).

These findings suggest that CONV farming practices are more strongly associated with the presence of ARGs compared to ABF systems, especially at the farm level. These findings are in line with previous meta-analyses, which observed reduced ARG levels in ABF systems compared to CONV ones (Nobrega et al., 2021; Ager et al., 2023). Notably, ARG levels in retail products appeared lower. This could partly be reflected due the smaller number of studies focused on retail samples. However, some studies reported inconsistent findings between products from CONV and ABF systems, while others reported no significant differences in ARG composition (Vikram et al., 2018; De Cesare et al., 2022). These inconsistencies highlight the need for more standardized, large-scale studies to better understand how farming practices influence ARG propagation.

However, targeted interventions such as on-farm biosecurity measures (e.g., enhanced sanitation, controlled animal flow), judicious feed and water management, and processing-stage decontamination (e.g., steam pasteurization, organic acid washes) are critical to reduce ARG levels before meat reaches consumers. The relationships between antibiotic use and resistance are still complex. They can vary widely depending on the specific antibiotic administration model, host, pathogen, detection methods used, sample source, study design, country-specific regulations, and all direct and indirect-environmental factors (Andersson et al., 2020; Larsson and Flach, 2022). This complexity was also reflected in our findings: studies targeting specific ARGs versus whole resistomes produced variable results.

However, despite these differences, ARGs were detected in both farming systems. Notably, 97% ($n = 36$) of studies reported ARG detection in ABF settings, suggesting that AMR is not solely driven by direct antibiotic use. Other factors, including contaminated animal feed, environmental exposure (air, water, and waste), poor farm management, and human activity, likely contribute to AMR spread (Gemedi et al., 2021; Mak et al., 2022). Together, the reviewed studies show that environmental reservoirs, farming practices, and microbial evolution each contribute to ARG dissemination. Our results closely mirror those of (Nobrega et al., 2021) and (Ager et al., 2023), reinforcing that on-farm antibiotic bans alone do not eliminate the resistome burden. We acknowledge that ARG prevalence is modulated by farm size, climate, biosecurity protocols, feed/water quality, and laboratory methods, and recommend future multivariable meta-regression to untangle these effects. Since multiple meta-analyses are finding the same patterns, we need large, coordinated studies using the same methods to tell apart the effects of farming system from environmental factors on AMR. Addressing AMR effectively will require a broader One Health approach that considers not just antimicrobial use, but also environmental contamination and farm management strategies.

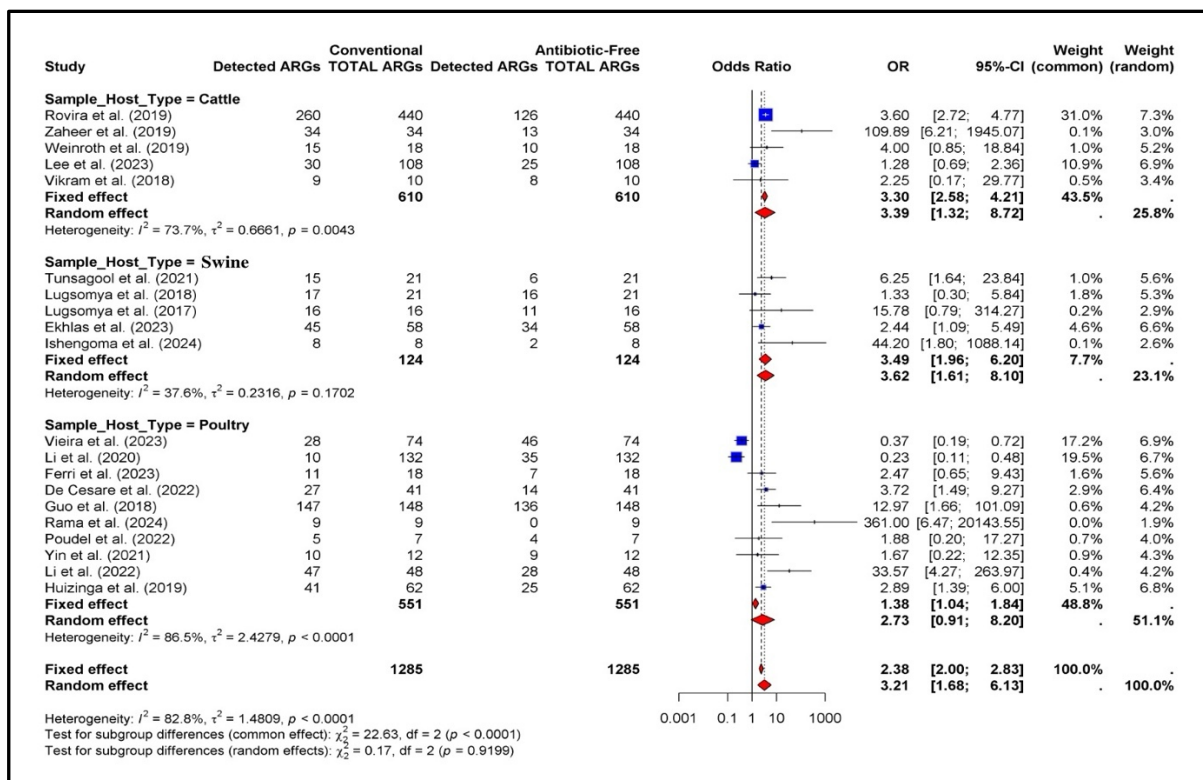


Figure 4. Forest plot summarizing the odds ratios (OR) for the detection of ARGs in CONV versus ABF systems across cattle, poultry, and pigs. Using a fixed-effects model, the pooled OR is 2.38 (95% CI: 2.00–2.83), and the random-effects model yields an OR of 3.21 (95% CI:

1.68–6.13), indicating that conventional systems have a significantly higher prevalence of ARGs. Significant heterogeneity was observed ($I^2 = 82.8\%$, $\tau^2 = 1.4809$, $p < 0.0001$). Subgroup analysis under the fixed-effects model showed significant differences among host types ($\chi^2 = 22.63$, $df = 2$, $p < 0.0001$), whereas the random-effects model did not reveal significant subgroup differences ($\chi^2 = 0.17$, $df = 2$, $p = 0.9199$). In this plot, the blue squares represent the individual study effect estimates and their weights, while the red diamond shows the overall pooled estimate with its confidence interval.

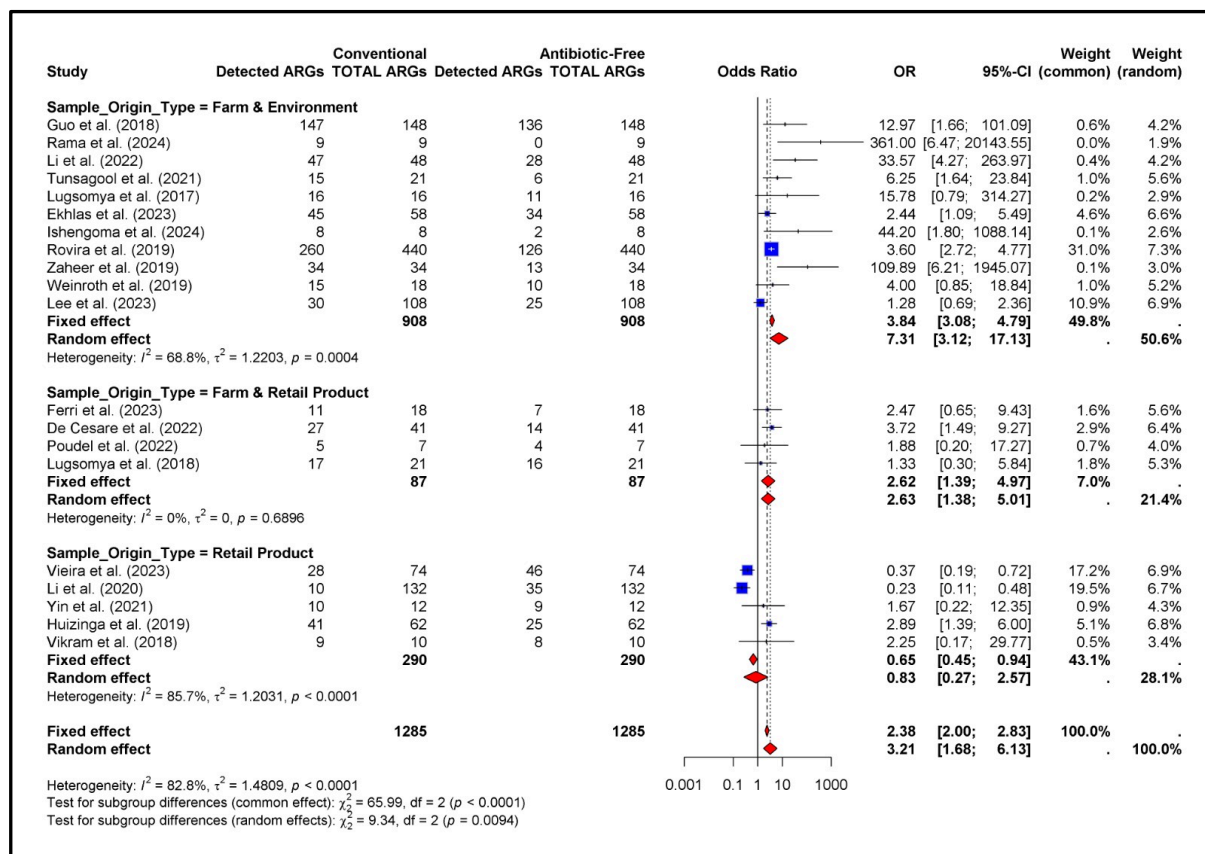


Figure 5. Forest plot comparing ARG prevalence based on sample origin, categorized as farm & environment, combined farm and retail products, and retail products only in CONV versus ABF systems. The fixed-effects model yields a pooled OR of 2.38 (95% CI: 2.00–2.83) and the random-effects model yielded an OR of 3.21 (95% CI: 1.68–6.13), reinforcing that conventional systems tend to have higher ARGs prevalence. Heterogeneity was high ($I^2 = 82.8\%$, $\tau^2 = 1.4809$, $p < 0.0001$). Subgroup analyses indicated that sample origin significantly influences ARG detection, with the common effect model yielding $\chi^2 = 65.99$ ($df = 2$, $p < 0.0001$) and the random effects model yielding $\chi^2 = 9.34$ ($df = 2$, $p = 0.0094$). In this plot, the blue squares represent the individual study effect estimates and their weights, while the red diamond shows the overall pooled estimate with its confidence interval.

4. Importance of AMR in a One Health concept

4.1. AMR at Farms:

Beyond farming practices, environmental factors also play a critical role in shaping AMR patterns. For example, agricultural soil acts as a natural reservoir for a variety of microorganisms. Hence, the application of pesticides, herbicides, insecticides, and antimicrobials exerts selective pressure on microbes inhabiting feed crops and soil, promoting multidrug and cross-resistance traits (Qiu et al., 2022). Additionally, the combination of antimicrobials with toxic metals such as copper further enhances this resistance (Jun et al., 2019). Moreover, the use of contaminated water and manure application in agriculture may further introduce ARGs in soil, water, and air. Indeed, manure-amended soils were found to have higher levels of ARGs than soils treated with chemical fertilizers (Tiedje et al., 2019; He et al., 2020). Beyond soil, contaminated animal feed, resulting from poor storage hygiene or exposure to wildlife (e.g., rodents, birds), can further introduce and spread ARGs (Auffret et al., 2017). Similarly, drinking water, even from private wells, can also serve as a vector for ARB transmission (Hayward et al., 2020; Alawi et al., 2024). Additionally, farm dust (airborne particles), migrating birds, rodents, pets, and insects are other components that may contribute to the spread of ARGs between farms and the environment (Gwenzi et al., 2021; Bai et al., 2022; Guardia et al., 2024) (**Figure 6**).

4.2. AMR in Meat Processing Plants:

Meat processing facilities serve as crucial hotspots for the emergence and dissemination of AMR. For instance, mixing animals from different farms during transportation and at lairage sites increases the risk of cross-contamination (Hazards et al., 2022). Additionally, procedures such as evisceration, defeathering, and de-hiding elevate initial microbial loads, resulting in further ARB and ARG contamination. Indeed, ARG abundance in a new meat processing plant increased significantly over 1.5 years, due to the transfer of ARB from animal carcasses to surfaces, tools, and drains, highlighting their role as reservoirs of ARGs (Cobo-Díaz et al., 2021). Despite sanitation measures, biofilms on processing surfaces may retain ARB, facilitating their spread throughout the entire food production chains, as demonstrated for several types of foods (De Filippis et al., 2021; Valentino et al., 2022; Sequino et al., 2024; Barcenilla et al., 2024; De Filippis et al., 2024; Valentino et al., 2023). Moreover, cleaning agents used for disinfection in processing environments apply a selective pressure on foodborne bacteria, potentially resulting in adaptive changes in their genomes, transcriptomes,

and proteomes, further enhancing their resistance to disinfectants and antibiotics (Marmion et al., 2022; Xiao et al., 2024). Therefore, the wastewater generated in food processing facilities has high levels of ARB and ARG, leading to the environmental contamination of other water bodies (Stošić et al., 2016) (**Figure 6**). Beyond the food production sector, the spread of AMR in the environment is influenced by a multitude of factors, including clinical settings, domestic practices, climate change, and various ecological and human-related activities. Indeed, AMR spread is a “One Health” issue, that summarizes well that the health of humans, animals, and the environment is strictly interconnected and should be addressed with a more comprehensive approach, tackling all the aspects involved.

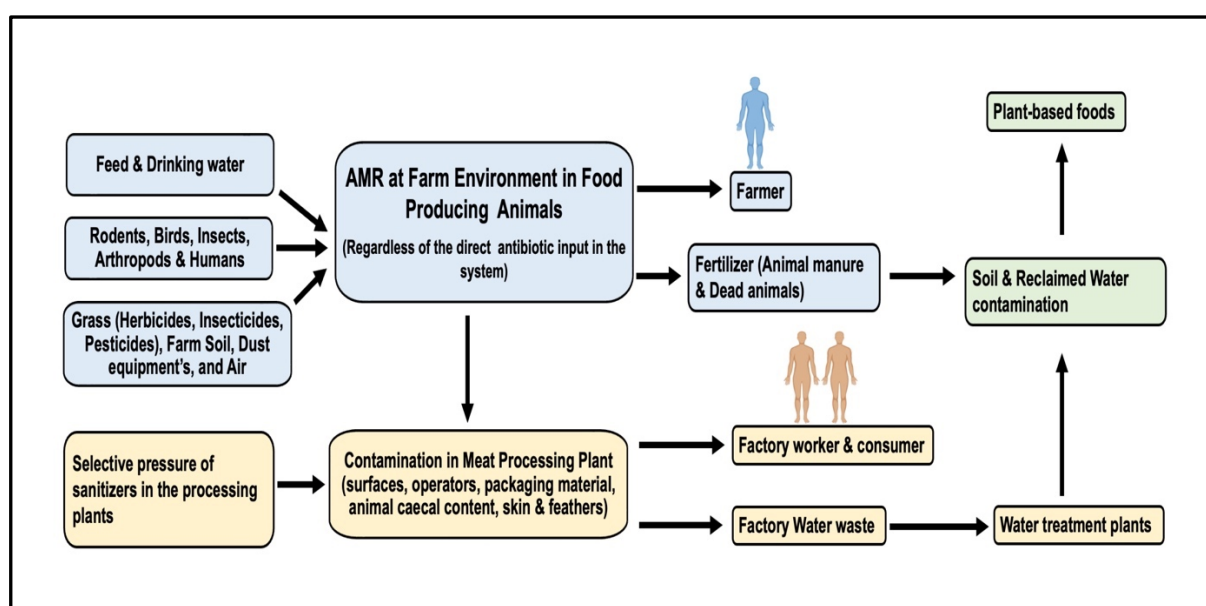


Figure 6. A schematic representation that illustrates the flow of antimicrobial resistance (AMR) among livestock animals, humans, and their associated environments. The light-blue pathway shows AMR flow related to livestock animals, farms, and their associated environments at the farm level, showing how different factors like rodents, insects, drinking water, herbicides, pesticides, and farm soil play their role in AMR development and propagation in food-producing animals. The light-yellow pathway indicates AMR flow associated with the environment and equipment within the processing plants, their retail products, and their effluents (e.g., water and solid waste). The light-green boxes represent AMR flow linked to environmental sources such as field soil, plant-based foods, underground and freshwater sources. This whole workflow demonstrates how AMR circulates among different contributors, irrespective of the direct introduction of antimicrobials into livestock farming. It also highlights the importance of the co-evolution of antimicrobial resistance genes (ARGs) in response to other selective pressures.

4.3. AMR in Health Settings:

The relationship between antibiotic administration in animal farming and the dissemination of AMR remains a subject of current debate. Nevertheless, various investigations suggest that the misuse of antibiotics in humans, along with environmental influences, could play a more crucial role (Mather et al., 2013). For instance, approximately 60.1% of cases involving extended-spectrum beta-lactamase-producing *E. coli* (relevant ARGs are CTX-M, TEM, and SHV families) and plasmid-mediated AmpC-producing (cephalosporinases) *E. coli* were associated with human-to-human transmission, especially in household and healthcare settings. In contrast, minimal contributions came from food (18.9%), companion animals (7.9%), farm animals (3.6%), and various environmental sources (2.6%) (Mughini-Gras et al., 2019). Furthermore, 63.5% of the estimated 671,689 AMR infections in EU and EEA countries were associated with transmission linked with healthcare settings (Cassini et al., 2019). This emphasizes the impact of clinical antibiotic use and human interactions in AMR onset and transmission, which frequently outweigh the impact of animal farming (**Figure 7**).

4.4. AMR in Household Settings:

Similarly, practices regarding household cleaning and the extensive use of personal care products are gaining acknowledgment as vital contributors to global AMR persistence. For instance, typical formulations in personal care products, such as those containing platinum-based compounds, exhibit antimicrobial capabilities by denaturing enzymes, destroying DNA, lysing cells, and generating reactive oxygen species (Stokes et al., 2024; Tahir et al., 2017). These chemical residues are washed away in greywater systems, where they interact with environmental bacteria and micropollutants, promoting ARB emergence (Pruden et al., 2018; Mim et al., 2024). Finally, also household animals may serve as reservoirs and vectors for the spread of antimicrobial resistance to humans (Bhat, 2021) (**Figure 7**).

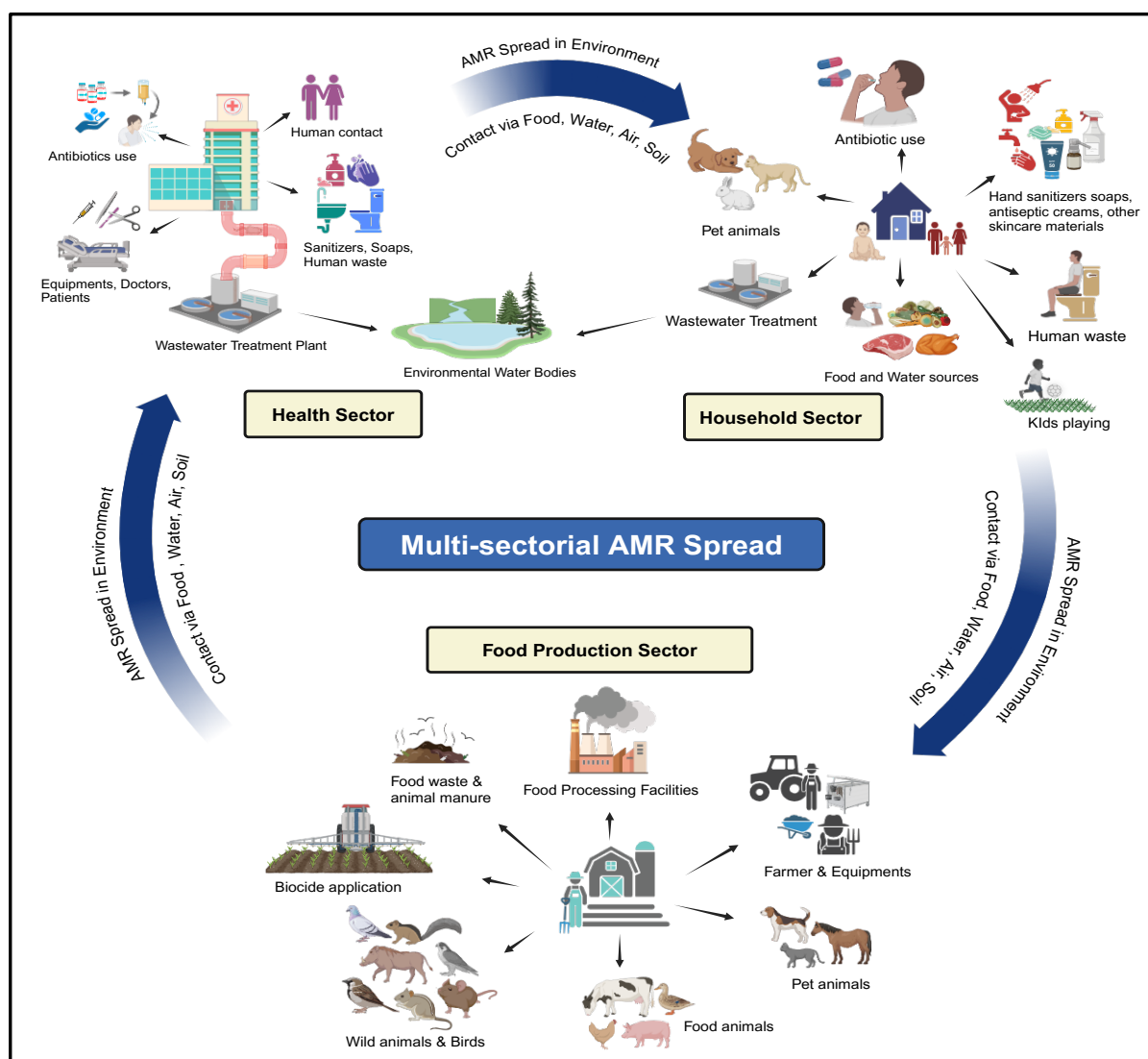


Figure 7. Overview of AMR flow across clinical, domestic, and agricultural systems. This scheme outlines a particular subset of the broad AMR landscape, illustrating the role of clinical treatments, household cleaning, and agricultural practices in the development of AMR. Created in BioRender. RAHMAN, A. (2025) <https://BioRender.com/m51i802>

4.5. AMR at Global Level:

In addition to the previously discussed factors, AMR is also influenced by a wide range of ecological and climate factors that extend beyond agricultural, hospital, and household contexts (Figure 8). For example, elevated temperatures, increased humidity, and higher pollution levels promote the proliferation of microbes and facilitate gene transfer. A 1 °C rise in average ambient temperature is associated with a 1.14-fold and a 1.06-fold increase, respectively, in the population-level prevalence of carbapenem-resistant *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* clinical isolates (Li et al., 2023). This elevated global temperature also mediates the

release of ARB from the thawing permafrost. A comprehensive review encompassing 574 studies revealed that 39% identified detrimental synergies between climate change and AMR, with 19% establishing a direct connection between climate change and the emergence of AMR, while 12% observed beneficial interactions between climate interventions and AMR control (Bavel et al., 2024). Human activities such as deforestation also increase the prevalence of ARGs in soils by exerting environmental selective pressures on un-exposed soil microbial communities (Lemos et al., 2021). Similarly, the combination of these activities with microplastic and chemical pollution further accelerates the evolution of ARGs (Rzymiski et al., 2024). For instance, a 1% rise in airborne particulate matter (PM_{2.5}) is related to a 0.7% increase in AMR (Natali, 2025). Likewise, global travel and trade play a substantial role in the transboundary spread of resistant strains (**Figure 8**) (Mendelsohn et al., 2023).

In addition to the environmental drivers, microbial evolution itself plays a key role in AMR persistence. Resistance can arise naturally through spontaneous mutations and spread through vertical and horizontal gene transfer, aided by mobile genetic elements like plasmids and transposons (Djordjevic et al., 2013). Moreover, co-selection mechanisms such as cross-resistance, co-resistance, and co-regulation allow bacteria to acquire multiple resistance traits even without direct antibiotic exposure (Murray et al., 2024). Together, these biological and environmental factors underscore the need for coordinated interventions, which are discussed in the following recommendations (**Figure 9**).

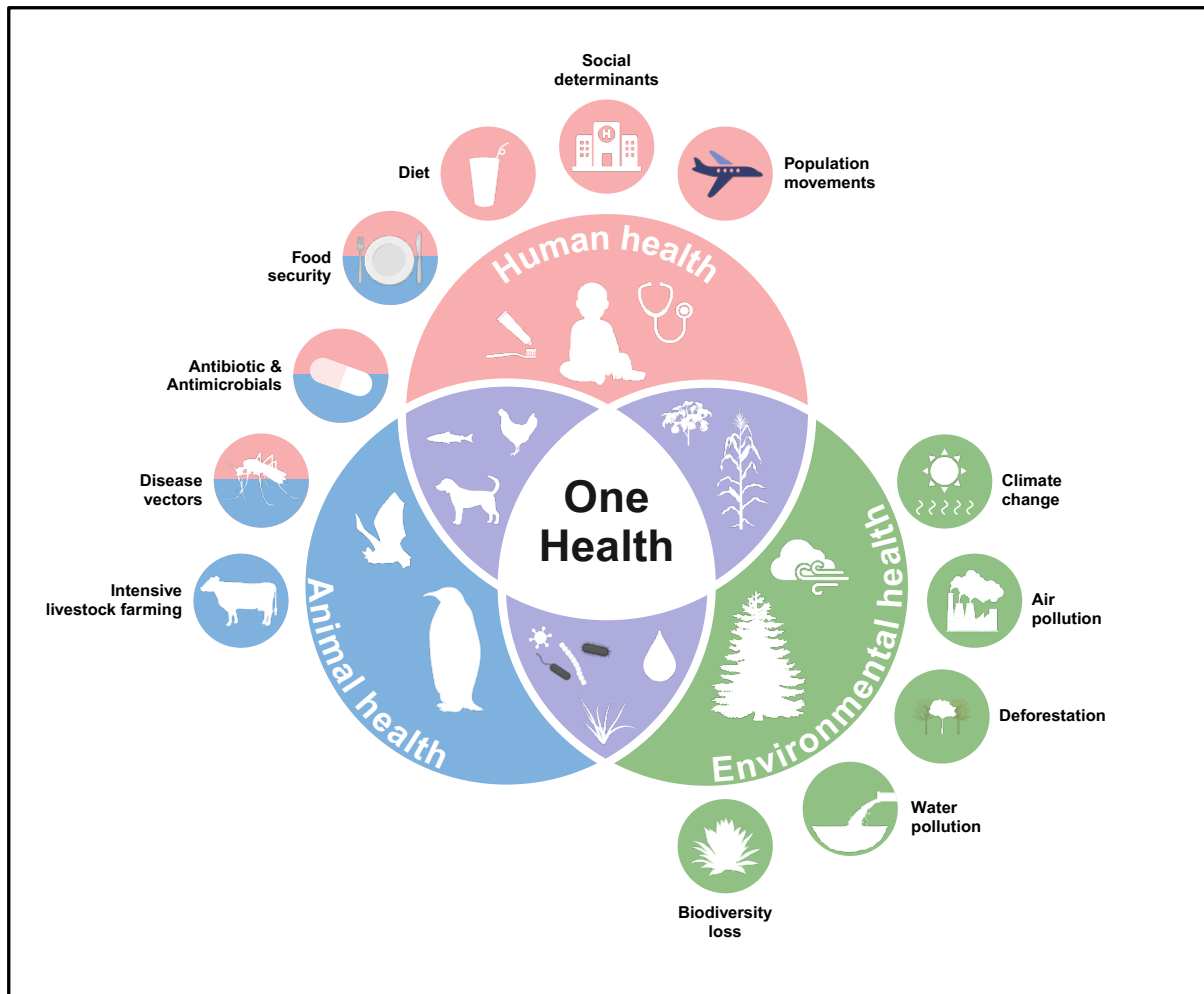


Figure 8. The One Health concept scheme showing how the health of people, animals, and the environment are all connected within the One Health framework. *Created in BioRender.* RAHMAN, A. (2025) <https://BioRender.com/e08j265>

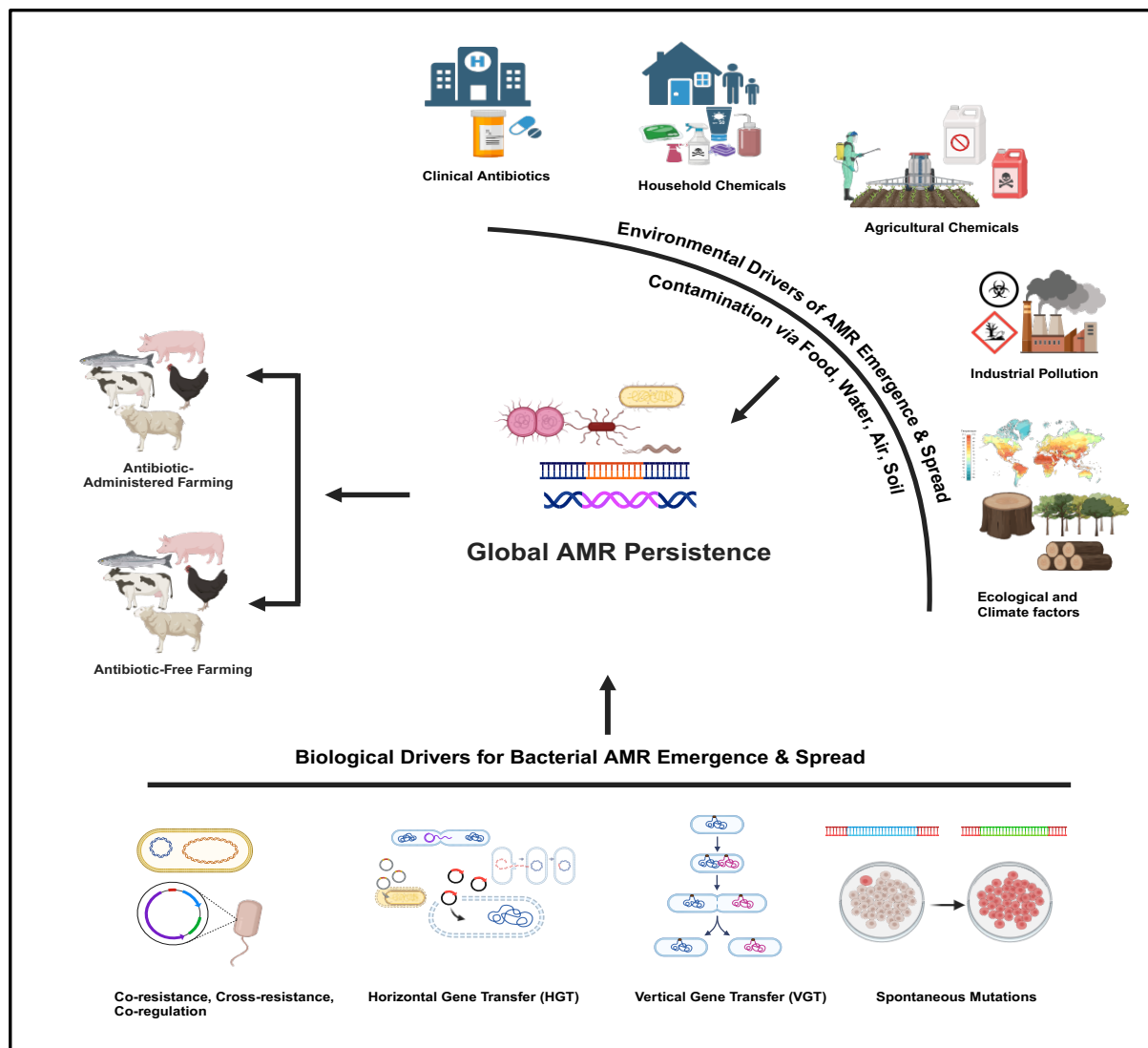


Figure 9. Visual summary of the environmental and biological drivers of persistent AMR in animal food systems. The figure illustrates how various sources including environmental contamination, microbial evolution, and cross-sectoral influences contribute to the persistence and spread of AMR. Created in BioRender by Rahman, A. (2025). <https://BioRender.com/r26z013>

5. Future Research Recommendations

5.1. Advancing Surveillance and Diagnostics

Comprehensive and real-time monitoring of AMR is crucial in various ecosystems to detect resistance trends in animals, humans, and the environment. Conventional monitoring is based on a restricted set of pathogens, often missing the emerging resistance threats. However, innovations in metagenomics, AI-enhanced predictive models, and machine learning can provide a proactive approach to detect emerging AMR hotspots prior to the onset of outbreaks (Lastra et al., 2024). Furthermore, misdiagnosis within both human and veterinary medicine plays a substantial role in the rise of AMR (Chan et al., 2020). The development of affordable, user-friendly, rapid, and reliable diagnostic tests is essential for decreasing dependence on broad-spectrum antibiotics and encouraging targeted antimicrobial treatments.

5.2. Mitigation Strategies at the Farm and Processing Levels

Prompt surveillance and instant isolation of infected animals can mitigate the AMR spread at the farm level. Additionally, decreasing stock density, increasing rearing space, and increasing farm biosecurity and herd management can further mitigate the spread of AMR (Dhaka et al., 2023). Post-slaughter washing of beef, pork, and lamb carcasses can effectively reduce contamination loads and improve meat hygiene at the initial stages of processing. Furthermore, the implementation of advanced on-site wastewater treatment, air filtration, and ventilation systems are essential to prevent the discharge of ARB and ARG into the environment. Similarly, the final meat products frequently contain spoilage microorganisms, pathogens, ARGs, and even antibiotic residues; however, studies on their effects on the human oral and gut microbiome following ingestion remain limited and insufficiently investigated. Although comprehensive cooking diminishes bacterial populations, the fate of ARGs or antibiotic residues during digestion, as well as the possible transmission of ARGs to the gut microbiome are still unclear.

5.3. Reducing Antibiotic Dependence, Exploring Alternatives and Public Awareness

Alternative treatments to traditional antibiotics, such as probiotics, prebiotics, bacteriophage therapy, and vaccinations, show promise in lowering infection rates and reducing antibiotic use. Furthermore, innovative conjugated antibiotic formulations may also enhance therapeutic efficacy against resistant microbes (Padilla and Nowick, 2025). Public involvement is also critical, yet it is frequently overlooked in AMR mitigation. Public education campaigns, using

the most popular social media platforms should educate the public about how their everyday lifestyle choices influence the global AMR spread.

5.4. Global Collaboration and Policy Integration

Considering the transboundary characteristics of AMR, public health agencies, environmental organizations, policymakers, and industry stakeholders need to collaborate on a global scale. Regulatory frameworks need to encompass a broader range of substances, including:

- Biocides, pesticides, heavy metals, and household chemicals.
- Enhanced wastewater management strategies aimed at minimizing ARG contamination in natural water systems.
- Strengthened data-sharing initiatives to facilitate coordinated global efforts in AMR control.

While ongoing surveillance and improved diagnostic capacity are essential for early detection of AMR trends, we recommend rebalancing funding priorities. Core surveillance should be maintained at a baseline level, enough to monitor emerging threats, while the bulk of financial resources should be redirected from this intensive surveillance and quantification of AMR toward solution-driven research. AMR is a well-documented problem; nevertheless, funding should now be focused on discovering novel solutions rather than sustaining the documentation.

6. Conclusions

This study systematically reviewed and meta-analyzed the presence of ARGs in CONV and ABF animal farming systems. ARGs were detected in both systems, with higher average counts in CONV farms. However, statistical differences in ARG prevalence across farming systems, host types, and sample origins were not significant. Subgroup analysis showed higher ARG presence in cattle and environmental samples, suggesting farm-level conditions may play a more critical role in ARG transmission than retail product exposure. However, this analysis is limited by the predominance of poultry studies, variability in study designs and ARG detection methods, and the lack of geographic metadata across the included research.

Notably, 97% of studies on ABF farms still reported the presence of ARGs, suggesting that reducing antibiotic use alone may not be sufficient to control AMR. Broader factors such as poor farm hygiene, environmental contamination, inadequate waste management, and microbial evolution likely contribute to its persistence. While minimizing antibiotic use in

animal farming has been a longstanding goal, the spread of AMR along the meat production chain involves many other drivers. Therefore, it is important to recognize that AMR is not solely caused by the overuse or misuse of antimicrobials in animals. Rather, AMR is a global issue that demands shared responsibility across agriculture, healthcare, households, and environmental sectors, particularly in the context of climate change.

These findings support the One Health narrative, where effective AMR control requires coordinated action across sectors. Targeted interventions at the farm and processing level, combined with policies addressing environmental and societal drivers, are urgently needed to reduce the risk of ARG transmission and protect public health.

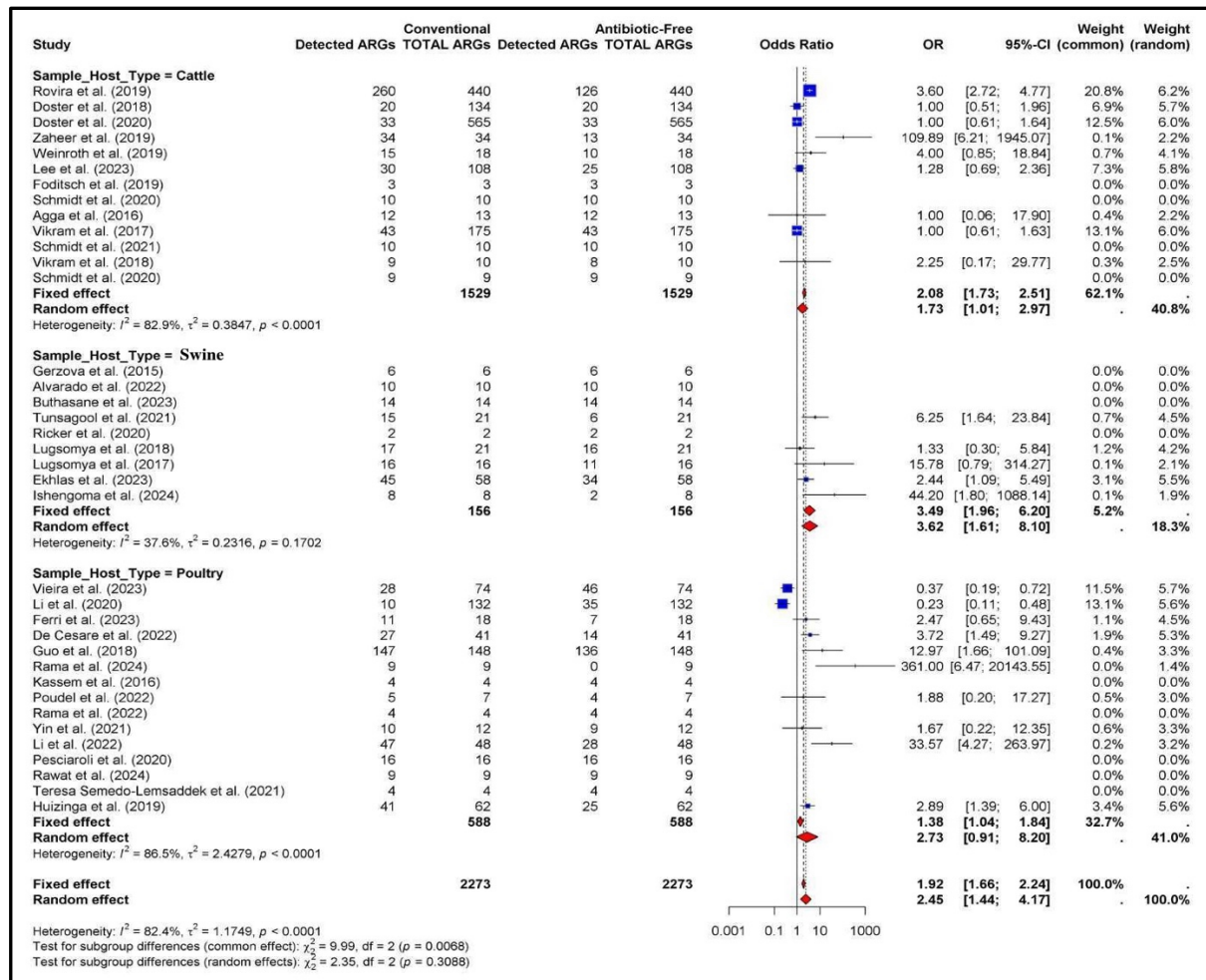


Figure S1. Forest plot summarizing the odds ratios (OR) for the detection of ARGs in CONV versus ABF systems across cattle, poultry, and pigs. Using a fixed-effects model, the pooled OR is 1.92 (95% CI: 1.66–2.24), and the random-effects model yields an OR of 2.45 (95% CI: 1.44–4.17), indicating that conventional systems have a significantly higher prevalence of ARGs. Significant heterogeneity was observed ($I^2 = 82.4\%$, $\tau^2 = 1.1749$, $p < 0.0001$). Subgroup analysis under the fixed-effects model showed significant differences among host types ($\chi^2 = 9.99$, $df = 2$, $p < 0.0068$), whereas the random-effects model did not reveal significant subgroup differences ($\chi^2 = 2.35$, $df = 2$, $p = 0.3088$). In this plot, the blue squares represent the individual study effect estimates and their weights, while the red diamond shows the overall pooled estimate with its confidence interval.

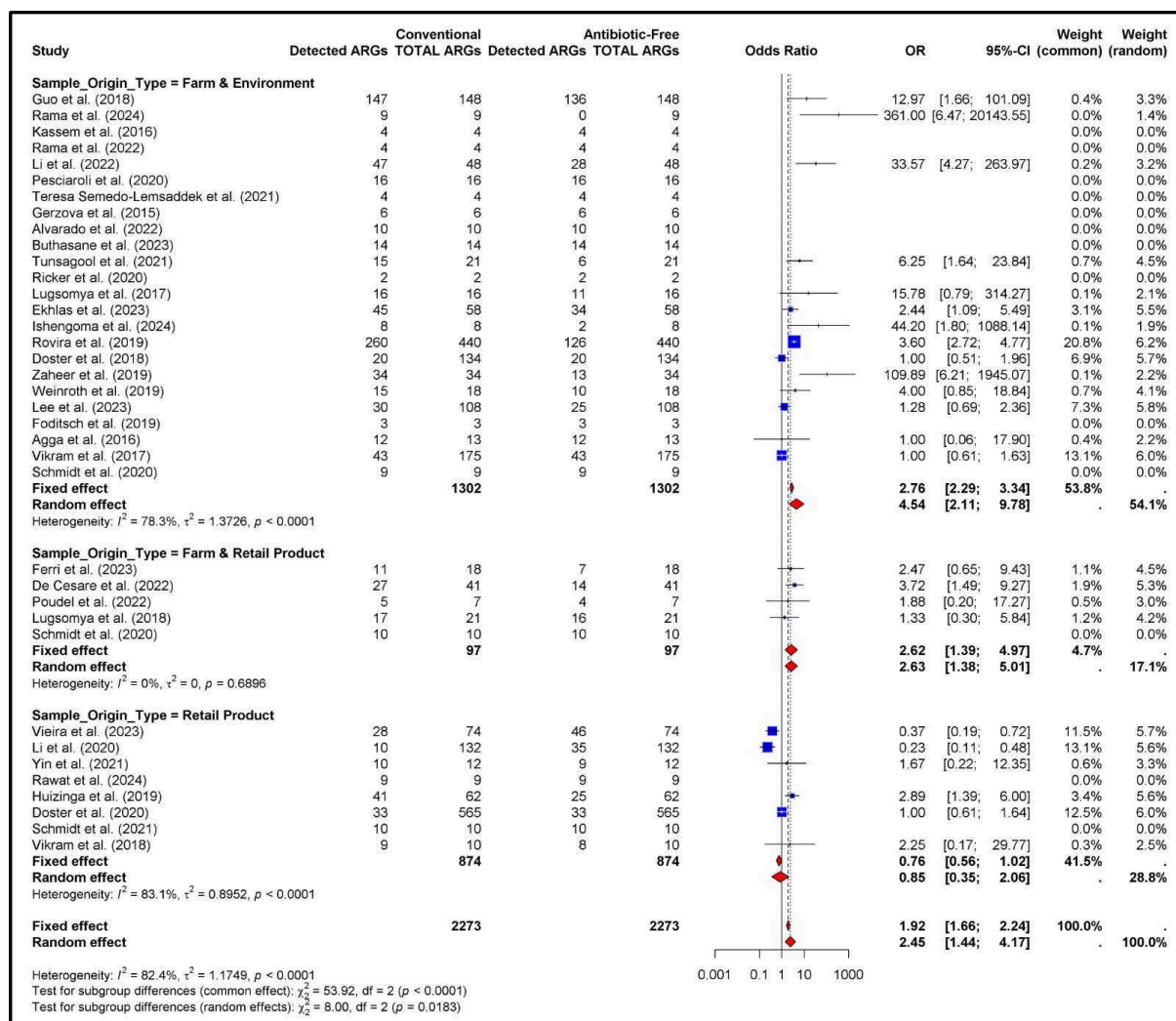


Figure S2. Forest plot comparing ARG prevalence based on sample origin, categorized as farm & environment, combined farm and retail products, and retail products only in CONV versus ABF systems. The fixed-effects model yields a pooled OR of 1.92 (95% CI: 1.66–2.24) and the random-effects model yielded an OR of 2.45 (95% CI: 1.44–4.17), reinforcing that conventional systems tend to have higher ARGs prevalence. Heterogeneity is high ($I^2 = 82.4\%$, $\tau^2 = 1.1749$, $p < 0.0001$). Subgroup analyses indicate that sample origin significantly influences ARG detection, with the common effect model reporting $\chi^2 = 53.92$ ($df = 2$, $p < 0.0001$) and the random effects model yielding $\chi^2 = 8.00$ ($df = 2$, $p = 0.0183$). In this plot, the blue squares represent the individual study effect estimates and their weights, while the red diamond shows the overall pooled estimate with its confidence interval.

References

1. Ager, E.O., Carvalho, T., Silva, E.M., Ricke, S.C., Hite, J.L., 2023. Global trends in antimicrobial resistance on organic and conventional farms. *Sci Rep* 13, 22608. <https://doi.org/10.1038/s41598-023-47862-7>
2. Alawi, M., Smyth, C., Drissner, D., Zimmerer, A., Leupold, D., Müller, D., Do, T.T., Velasco-Torrijos, T., Walsh, F., 2024. Private and well drinking water are reservoirs for antimicrobial resistant bacteria. *npj Antimicrob Resist* 2, 1–16. <https://doi.org/10.1038/s44259-024-00024-9>
3. Alvarado, A.C., Chekabab, S.M., Predicala, B.Z., Korber, D.R., 2022. Impact of Raised without Antibiotics Measures on Antimicrobial Resistance and Prevalence of Pathogens in Sow Barns. *Antibiotics* 11, 1221. <https://doi.org/10.3390/antibiotics11091221>
4. Andersson, D.I., Balaban, N.Q., Baquero, F., Courvalin, P., Glaser, P., Gophna, U., Kishony, R., Molin, S., Tønjum, T., 2020. Antibiotic resistance: turning evolutionary principles into clinical reality. *FEMS Microbiol Rev* 44, 171–188. <https://doi.org/10.1093/femsre/fuaa001>
5. Auffret, M.D., Dewhurst, R.J., Duthie, C.-A., Rooke, J.A., John Wallace, R., Freeman, T.C., Stewart, R., Watson, M., Roehe, R., 2017. The rumen microbiome as a reservoir of antimicrobial resistance and pathogenicity genes is directly affected by diet in beef cattle. *Microbiome* 5, 159. <https://doi.org/10.1186/s40168-017-0378-z>
6. Bai, H., He, L.-Y., Wu, D.-L., Gao, F.-Z., Zhang, M., Zou, H.-Y., Yao, M.-S., Ying, G.-G., 2022. Spread of airborne antibiotic resistance from animal farms to the environment: Dispersal pattern and exposure risk. *Environment International* 158, 106927. <https://doi.org/10.1016/j.envint.2021.106927>
7. Barcenilla, C., Cobo-Díaz, J.F., Puente, A., Valentino, V., De Filippis, F., Ercolini, D., Carlino, N., Pinto, F., Segata, N., Prieto, M., López, M., Alvarez-Ordóñez, A., 2024. In-depth characterization of food and environmental microbiomes across different meat processing plants. *Microbiome* 12, 199. <https://doi.org/10.1186/s40168-024-01856-3>
8. Bavel, B. van, Berrang-Ford, L., Moon, K., Gudda, F., Thornton, A.J., Robinson, R.F.S., King, R., 2024. Intersections between climate change and antimicrobial resistance: a systematic scoping review. *The Lancet Planetary Health* 8, e1118–e1128. [https://doi.org/10.1016/S2542-5196\(24\)00273-0](https://doi.org/10.1016/S2542-5196(24)00273-0)

9. Bhat, A.H., 2021. Bacterial zoonoses transmitted by household pets and as reservoirs of antimicrobial resistant bacteria. *Microbial Pathogenesis* 155, 104891. <https://doi.org/10.1016/j.micpath.2021.104891>
10. Brasil, 2009. Ministry of Agriculture, Livestock and Food Supply. MARKETING BY THIRD PARTIES PROHIBITED 10.
11. Buthasane, P., Roytrakul, S., Phaonakrop, N., Tunsagool, P., Buthasane, W., Am-in, N., Suriyaphol, G., 2023. Metaproteomic Analysis of Gut Resistome in the Cecal Microbiota of Fattening Pigs Raised without Antibiotics. *Microbiology Spectrum* 11, e02223-23. <https://doi.org/10.1128/spectrum.02223-23>
12. Cassini, A., Högberg, L.D., Plachouras, D., Quattrocchi, A., Hoxha, A., Simonsen, G.S., Colomb-Cotin, M., Kretzschmar, M.E., Devleeschauwer, B., Cecchini, M., Ouakrim, D.A., Oliveira, T.C., Struelens, M.J., Suetens, C., Monnet, D.L., Strauss, R., Mertens, K., Struyf, T., Catry, B., Latour, K., Ivanov, I.N., Dobrev, E.G., Andrašević, A.T., Soprek, S., Budimir, A., Paphitou, N., Žemlicková, H., Olsen, S.S., Sönksen, U.W., Märtin, P., Ivanova, M., Lyytikäinen, O., Jalava, J., Coignard, B., Eckmanns, T., Sin, M.A., Haller, S., Daikos, G.L., Gikas, A., Tsiodras, S., Kontopidou, F., Tóth, Á., Hajdu, Á., Guðlaugsson, Ó., Kristinsson, K.G., Murchan, S., Burns, K., Pezzotti, P., Gagliotti, C., Dumpis, U., Liuimienė, A., Perrin, M., Borg, M.A., Greeff, S.C. de, Monen, J.C., Koek, M.B., Elstrøm, P., Zabicka, D., Deptula, A., Hryniewicz, W., Caniça, M., Nogueira, P.J., Fernandes, P.A., Manageiro, V., Popescu, G.A., Serban, R.I., Schréterová, E., Litvová, S., Štefkovicová, M., Kolman, J., Klavs, I., Korošec, A., Aracil, B., Asensio, A., Pérez-Vázquez, M., Billström, H., Larsson, S., Reilly, J.S., Johnson, A., Hopkins, S., 2019. Attributable deaths and disability-adjusted life-years caused by infections with antibiotic-resistant bacteria in the EU and the European Economic Area in 2015: a population-level modelling analysis. *The Lancet Infectious Diseases* 19, 56–66. [https://doi.org/10.1016/S1473-3099\(18\)30605-4](https://doi.org/10.1016/S1473-3099(18)30605-4)
13. Chan, K.W., Bard, A.M., Adam, K.E., Rees, G.M., Morgans, L., Cresswell, L., Hinchliffe, S., Barrett, D.C., Reyher, K.K., Buller, H., 2020. Diagnostics and the challenge of antimicrobial resistance: a survey of UK livestock veterinarians' perceptions and practices. *Vet Rec* 187, e125. <https://doi.org/10.1136/vr.105822>
14. Cobo-Díaz, J.F., Alvarez-Molina, A., Alexa, E.A., Walsh, C.J., Mencía-Ares, O., Puente-Gómez, P., Likotrafiti, E., Fernández-Gómez, P., Prieto, B., Crispie, F., Ruiz, L., González-Raurich, M., López, M., Prieto, M., Cotter, P., Alvarez-Ordóñez, A., 2021. Microbial colonization and resistome dynamics in food processing environments of a

- newly opened pork cutting industry during 1.5 years of activity. *Microbiome* 9, 204. <https://doi.org/10.1186/s40168-021-01131-9>
15. De Cesare, A., Oliveri, C., Lucchi, A., Savini, F., Manfreda, G., Sala, C., 2022. Pilot Study on Poultry Meat from Antibiotic Free and Conventional Farms: Can Metagenomics Detect Any Difference? *Foods* 11, 249. <https://doi.org/10.3390/foods11030249>
16. De Filippis, F., Valentino, V., Alvarez-Ordóñez, A., Cotter, P.D., Ercolini, D., 2021. Environmental microbiome mapping as a strategy to improve quality and safety in the food industry. *Current Opinion in Food Science* 38, 168–176. <https://doi.org/10.1016/j.cofs.2020.11.012>
17. De Filippis, F., Valentino, V., Yap, M., Cabrera-Rubio, R., Barcenilla, C., Carlino, N., Cobo-Díaz, J.F., Quijada, N.M., Calvete-Torre, I., Ruas-Madiedo, P., Sabater, C., Sequino, G., Pasolli, E., Wagner, M., Margolles, A., Segata, N., Álvarez-Ordóñez, A., Cotter, P.D., Ercolini, D., 2024. Microbiome mapping in dairy industry reveals new species and genes for probiotic and bioprotective activities. *npj Biofilms Microbiomes* 10, 1–16. <https://doi.org/10.1038/s41522-024-00541-5>
18. de la Lastra, J.M.P., Wardell, S.J.T., Pal, T., de la Fuente-Nunez, C., Pletzer, D., 2024. From Data to Decisions: Leveraging Artificial Intelligence and Machine Learning in Combating Antimicrobial Resistance – a Comprehensive Review. *J Med Syst* 48, 71. <https://doi.org/10.1007/s10916-024-02089-5>
19. Dhaka, P., Chantziaras, I., Vijay, D., Bedi, J.S., Makovska, I., Biebaut, E., Dewulf, J., 2023. Can Improved Farm Biosecurity Reduce the Need for Antimicrobials in Food Animals? A Scoping Review. *Antibiotics* 12, 893. <https://doi.org/10.3390/antibiotics12050893>
20. Djordjevic, S.P., Stokes, H.W., Roy Chowdhury, P., 2013. Mobile elements, zoonotic pathogens and commensal bacteria: conduits for the delivery of resistance genes into humans, production animals and soil microbiota. *Front. Microbiol.* 4, 86. <https://doi.org/10.3389/fmicb.2013.00086>
21. Doster, E., Rovira, P., Noyes, N.R., Burgess, B.A., Yang, X., Weinroth, M.D., Lakin, S.M., Dean, C.J., Linke, L., Magnuson, R., Jones, K.I., Boucher, C., Ruiz, J., Belk, K.E., Morley, P.S., 2018. Investigating Effects of Tulathromycin Metaphylaxis on the Fecal Resistome and Microbiome of Commercial Feedlot Cattle Early in the Feeding Period. *Front. Microbiol.* 9. <https://doi.org/10.3389/fmicb.2018.01715>

22. Gemed, B.A., Assefa, A., Jaleta, M.B., Amenu, K., Wieland, B., 2021. Antimicrobial resistance in Ethiopia: A systematic review and meta-analysis of prevalence in foods, food handlers, animals, and the environment. *One Health* 13, 100286. <https://doi.org/10.1016/j.onehlt.2021.100286>
23. Gerzova, L., Babak, V., Sedlar, K., Faldynova, M., Videnska, P., Cejkova, D., Jensen, A.N., Denis, M., Kerouanton, A., Ricci, A., Cibin, V., Österberg, J., Rychlik, I., 2015. Characterization of Antibiotic Resistance Gene Abundance and Microbiota Composition in Feces of Organic and Conventional Pigs from Four EU Countries. *PLOS ONE* 10, e0132892. <https://doi.org/10.1371/journal.pone.0132892>
24. Guardia, T., Varriale, L., Minichino, A., Balestrieri, R., Mastronardi, D., Russo, T.P., Dipineto, L., Fioretti, A., Borrelli, L., 2024. Wild birds and the ecology of antimicrobial resistance: an approach to monitoring. *The Journal of Wildlife Management* 88, e22588. <https://doi.org/10.1002/jwmg.22588>
25. Guo, X., Stedtfeld, R.D., Hedman, H., Eisenberg, J.N.S., Trueba, G., Yin, D., Tiedje, J.M., Zhang, L., 2018. Antibiotic Resistome Associated with Small-Scale Poultry Production in Rural Ecuador. *Environ. Sci. Technol.* 52, 8165–8172. <https://doi.org/10.1021/acs.est.8b01667>
26. Gwenzi, W., Chaukura, N., Muisa-Zikali, N., Teta, C., Musvuugwa, T., Rzymiski, P., Abia, A.L.K., 2021. Insects, Rodents, and Pets as Reservoirs, Vectors, and Sentinels of Antimicrobial Resistance. *Antibiotics* 10, 68. <https://doi.org/10.3390/antibiotics10010068>
27. Hayward, C., Ross, K.E., Brown, M.H., Whiley, H., 2020. Water as a Source of Antimicrobial Resistance and Healthcare-Associated Infections. *Pathogens* 9, 667. <https://doi.org/10.3390/pathogens9080667>
28. Hazards (BIOHAZ), E.P. on B., Koutsoumanis, K., Allende, A., Álvarez-Ordóñez, A., Bolton, D., Bover-Cid, S., Chemaly, M., Davies, R., De Cesare, A., Herman, L., Hilbert, F., Lindqvist, R., Nauta, M., Ru, G., Simmons, M., Skandamis, P., Suffredini, E., Argüello-Rodríguez, H., Dohmen, W., Magistrali, C.F., Padalino, B., Tenhagen, B.-A., Threlfall, J., García-Fierro, R., Guerra, B., Liébana, E., Stella, P., Peixe, L., 2022. Transmission of antimicrobial resistance (AMR) during animal transport. *EFSA Journal* 20, e07586. <https://doi.org/10.2903/j.efsa.2022.7586>
29. He, Y., Yuan, Q., Mathieu, J., Stadler, L., Senehi, N., Sun, R., Alvarez, P.J.J., 2020. Antibiotic resistance genes from livestock waste: occurrence, dissemination, and treatment. *npj Clean Water* 3, 1–11. <https://doi.org/10.1038/s41545-020-0051-0>

30. Huizinga, P., Bergh, M.K. den, Rossen, J.W., Willemsen, I., Verhulst, C., Savelkoul, P.H.M., Friedrich, A.W., García-Cobos, S., Kluytmans, J., 2019. Decreasing prevalence of contamination with extended-spectrum beta-lactamase-producing Enterobacteriaceae (ESBL-E) in retail chicken meat in the Netherlands. *PLOS ONE* 14, e0226828. <https://doi.org/10.1371/journal.pone.0226828>
31. Ishengoma, V.L., Amachawadi, R.G., Tokach, M.D., Kang, Q., Goodband, R.D., DeRouchey, J., Woodworth, J., Nagaraja, T.G., 2024. The Impact of In-Water vs. In-Feed Chlortetracycline and Tiamulin Administration in Piglets on the Fecal Prevalence and Antimicrobial Resistance of Salmonella. *Applied Microbiology* 4, 297–311. <https://doi.org/10.3390/applmicrobiol4010020>
32. Jun, H., Kurenbach, B., Aitken, J., Wasa, A., Remus-Emsermann, M.N., Godsoe, W., Heinemann, J.A., 2019. Effects of sub-lethal concentrations of copper ammonium acetate, pyrethrins and atrazine on the response of *Escherichia coli* to antibiotics. *F1000Research* 8, 32. <https://doi.org/10.12688/f1000research.17652.1>
33. Larsson, D.G.J., Flach, C.-F., 2022. Antibiotic resistance in the environment. *Nat Rev Microbiol* 20, 257–269. <https://doi.org/10.1038/s41579-021-00649-x>
34. Lemos, L.N., Pedrinho, A., Vasconcelos, A.T.R. de, Tsai, S.M., Mendes, L.W., 2021. Amazon deforestation enriches antibiotic resistance genes. *Soil Biology and Biochemistry* 153, 108110. <https://doi.org/10.1016/j.soilbio.2020.108110>
35. Li, S., Mann, D.A., Zhang, S., Qi, Y., Meinersmann, R.J., Deng, X., 2020. Microbiome-Informed Food Safety and Quality: Longitudinal Consistency and Cross-Sectional Distinctiveness of Retail Chicken Breast Microbiomes. *mSystems* 5, 1128. <https://doi.org/10.1128/msystems.00589-20>
36. Li, W., Liu, C., Ho, H.C., Shi, L., Zeng, Y., Yang, X., Huang, Q., Pei, Y., Huang, C., Yang, L., 2023. Association between antibiotic resistance and increasing ambient temperature in China: an ecological study with nationwide panel data. *The Lancet Regional Health – Western Pacific* 30. <https://doi.org/10.1016/j.lanwpc.2022.100628>
37. Li, Y., Ed-Dra, A., Tang, B., Kang, X., Müller, A., Kehrenberg, C., Jia, C., Pan, H., Yang, H., Yue, M., 2022. Higher tolerance of predominant *Salmonella* serovars circulating in the antibiotic-free feed farms to environmental stresses. *Journal of Hazardous Materials* 438, 129476. <https://doi.org/10.1016/j.jhazmat.2022.129476>
38. Mak, P.H.W., Rehman, M.A., Kiarie, E.G., Topp, E., Diarra, M.S., 2022. Production systems and important antimicrobial resistant-pathogenic bacteria in poultry: a review.

- Journal of Animal Science and Biotechnology 13, 148. <https://doi.org/10.1186/s40104-022-00786-0>
39. Manyi-Loh, C., Mamphweli, S., Meyer, E., Okoh, A., 2018. Antibiotic Use in Agriculture and Its Consequential Resistance in Environmental Sources: Potential Public Health Implications. *Molecules* 23, 795. <https://doi.org/10.3390/molecules23040795>
40. Marmion, M., Macori, G., Ferone, M., Whyte, P., Scannell, A.G.M., 2022. Survive and thrive: Control mechanisms that facilitate bacterial adaptation to survive manufacturing-related stress. *International Journal of Food Microbiology* 368, 109612. <https://doi.org/10.1016/j.ijfoodmicro.2022.109612>
41. Mather, A.E., Reid, S.W.J., Maskell, D.J., Parkhill, J., Fookes, M.C., Harris, S.R., Brown, D.J., Coia, J.E., Mulvey, M.R., Gilmour, M.W., Petrovska, L., de Pinna, E., Kuroda, M., Akiba, M., Izumiya, H., Connor, T.R., Suchard, M.A., Lemey, P., Mellor, D.J., Haydon, D.T., Thomson, N.R., 2013. Distinguishable Epidemics of Multidrug-Resistant *Salmonella* Typhimurium DT104 in Different Hosts. *Science* 341, 1514–1517. <https://doi.org/10.1126/science.1240578>
42. Mendelsohn, E., Ross, N., Zambrana-Torrel, C., Van Boeckel, T.P., Laxminarayan, R., Daszak, P., 2023. Global patterns and correlates in the emergence of antimicrobial resistance in humans. *Proceedings of the Royal Society B: Biological Sciences* 290, 20231085. <https://doi.org/10.1098/rspb.2023.1085>
43. Mim, M.F., Sikder, M.H., Chowdhury, M.Z.H., Bhuiyan, A.-U.-A., Zinan, N., Islam, S.M.N., 2024. The dynamic relationship between skin microbiomes and personal care products: A comprehensive review. *Heliyon* 10. <https://doi.org/10.1016/j.heliyon.2024.e34549>
44. Mughini-Gras, L., Dorado-García, A., Duijkeren, E. van, Bunt, G. van den, Dierikx, C.M., Bonten, M.J.M., Bootsma, M.C.J., Schmitt, H., Hald, T., Evers, E.G., Koeijer, A. de, Pelt, W. van, Franz, E., Mevius, D.J., Heederik, D.J.J., 2019. Attributable sources of community-acquired carriage of *Escherichia coli* containing β -lactam antibiotic resistance genes: a population-based modelling study. *The Lancet Planetary Health* 3, e357–e369. [https://doi.org/10.1016/S2542-5196\(19\)30130-5](https://doi.org/10.1016/S2542-5196(19)30130-5)
45. Murray, C.J.L., Ikuta, K.S., Sharara, F., Swetschinski, L., Aguilar, G.R., Gray, A., Han, C., Bisignano, C., Rao, P., Wool, E., Johnson, S.C., Browne, A.J., Chipeta, M.G., Fell, F., Hackett, S., Haines-Woodhouse, G., Hamadani, B.H.K., Kumaran, E.A.P., McManigal, B., Achalapong, S., Agarwal, R., Akech, S., Albertson, S., Amuasi, J.,

- Andrews, J., Aravkin, A., Ashley, E., Babin, F.-X., Bailey, F., Baker, S., Basnyat, B., Bekker, A., Bender, R., Berkley, J.A., Bethou, A., Bielicki, J., Boonkasidecha, S., Bukosia, J., Carvalho, C., Castañeda-Orjuela, C., Chansamouth, V., Chaurasia, S., Chiurchiù, S., Chowdhury, F., Donatien, R.C., Cook, A.J., Cooper, B., Cressey, T.R., Criollo-Mora, E., Cunningham, M., Darboe, S., Day, N.P.J., Luca, M.D., Dokova, K., Dramowski, A., Dunachie, S.J., Bich, T.D., Eckmanns, T., Eibach, D., Emami, A., Feasey, N., Fisher-Pearson, N., Forrest, K., Garcia, C., Garrett, D., Gastmeier, P., Giref, A.Z., Greer, R.C., Gupta, V., Haller, S., Haselbeck, A., Hay, S.I., Holm, M., Hopkins, S., Hsia, Y., Iregbu, K.C., Jacobs, J., Jarovsky, D., Javanmardi, F., Jenney, A.W.J., Khorana, M., Khusuwan, S., Kissoon, N., Kobeissi, E., Kostyaney, T., Krapp, F., Krumkamp, R., Kumar, A., Kyu, H.H., Lim, C., Lim, K., Limmathurotsakul, D., Loftus, M.J., Lunn, M., Ma, J., Manoharan, A., Marks, F., May, J., Mayxay, M., Mturi, N., Munera-Huertas, T., Musicha, P., Musila, L.A., Mussi-Pinhata, M.M., Naidu, R.N., Nakamura, T., Nanavati, R., Nangia, S., Newton, P., Ngoun, C., Novotney, A., Nwakanma, D., Obiero, C.W., Ochoa, T.J., Olivas-Martinez, A., Olliaro, P., Ooko, E., Ortiz-Brizuela, E., Ounchanum, P., Pak, G.D., Paredes, J.L., Peleg, A.Y., Perrone, C., Phe, T., Phommasone, K., Plakkal, N., Ponce-de-Leon, A., Raad, M., Ramdin, T., Rattanaovong, S., Riddell, A., Roberts, T., Robotham, J.V., Roca, A., Rosenthal, V.D., Rudd, K.E., Russell, N., Sader, H.S., Saengchan, W., Schnall, J., Scott, J.A.G., Seekaew, S., Sharland, M., Shivamallappa, M., Sifuentes-Osornio, J., Simpson, A.J., Steenkeste, N., Stewardson, A.J., Stoeva, T., Tasak, N., Thaiprakong, A., Thwaites, G., Tigoi, C., Turner, C., Turner, P., Doorn, H.R. van, Velaphi, S., Vongpradith, A., Vongsouvath, M., Vu, H., Walsh, T., Walson, J.L., Waner, S., Wangrangsimakul, T., Wannapinij, P., Wozniak, T., Sharma, T.E.M.W.Y., Yu, K.C., Zheng, P., Sartorius, B., Lopez, A.D., Stergachis, A., Moore, C., Dolecek, C., Naghavi, M., 2022. Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis. *The Lancet* 399, 629–655. [https://doi.org/10.1016/S0140-6736\(21\)02724-0](https://doi.org/10.1016/S0140-6736(21)02724-0)
46. Murray, L.M., Hayes, A., Snape, J., Kasprzyk-Hordern, B., Gaze, W.H., Murray, A.K., 2024. Co-selection for antibiotic resistance by environmental contaminants. *npj Antimicrob Resist* 2, 1–13. <https://doi.org/10.1038/s44259-024-00026-7>
47. Natali, I., 2025. Invisible Threat: How Airborne Pollution Fuels Antimicrobial Resistance in the EU [WWW Document]. URL <http://tse-fr.eu/pub/130132> (accessed 2.21.25).

48. Nobrega, D.B., Tang, K.L., Caffrey, N.P., De Buck, J., Cork, S.C., Ronksley, P.E., Polachek, A.J., Ganshorn, H., Sharma, N., Kastelic, J.P., Kellner, J.D., Ghali, W.A., Barkema, H.W., 2021. Prevalence of antimicrobial resistance genes and its association with restricted antimicrobial use in food-producing animals: a systematic review and meta-analysis. *Journal of Antimicrobial Chemotherapy* 76, 561–575. <https://doi.org/10.1093/jac/dkaa443>
49. O'Neill, J., 2016. Tackling drug-resistant infections globally: final report and recommendations.
50. Padilla, M.S.T.L., Nowick, J.S., 2025. Vancomycin–Teixobactin Conjugates. *J. Am. Chem. Soc.* 147, 6343–6348. <https://doi.org/10.1021/jacs.4c17175>
51. Pruden, A., Alcalde, R.E., Alvarez, P.J.J., Ashbolt, N., Bischel, H., Capiro, N.L., Crossette, E., Frigon, D., Grimes, K., Haas, C.N., Ikuma, K., Kappell, A., LaPara, T., Kimbell, L., Li, M., Li, X., McNamara, P., Seo, Y., Sobsey, M.D., Sozzi, E., Navab-Daneshmand, T., Raskin, L., Riquelme, M.V., Vikesland, P., Wigginton, K., Zhou, Z., 2018. An Environmental Science and Engineering Framework for Combating Antimicrobial Resistance. *Environmental Engineering Science* 35, 1005–1011. <https://doi.org/10.1089/ees.2017.0520>
52. QIMA/WQS [WWW Document], n.d. URL <https://wqs.com.br/> (accessed 2.17.25).
53. Qiu, D., Ke, M., Zhang, Q., Zhang, F., Lu, T., Sun, L., Qian, H., 2022. Response of microbial antibiotic resistance to pesticides: An emerging health threat. *Science of The Total Environment* 850, 158057. <https://doi.org/10.1016/j.scitotenv.2022.158057>
54. Rzymiski, P., Gwenzi, W., Poniedziałek, B., Mangul, S., Fal, A., 2024. Climate warming, environmental degradation and pollution as drivers of antibiotic resistance. *Environmental Pollution* 346, 123649. <https://doi.org/10.1016/j.envpol.2024.123649>
55. Schmidt, J.W., Vikram, A., Doster, E., Thomas, K., Weinroth, M.D., Parker, J., Hanes, A., Geornaras, I., Morley, P.S., Belk, K.E., Wheeler, T.L., Arthur, T.M., 2021. Antimicrobial Resistance in U.S. Retail Ground Beef with and without Label Claims Regarding Antibiotic Use. *Journal of Food Protection* 84, 827–842. <https://doi.org/10.4315/JFP-20-376>
56. Sequino, G., Cobo-Diaz, J.F., Valentino, V., Tassou, C., Volpe, S., Torrieri, E., Nychas, G.-J., Álvarez Ordóñez, A., Ercolini, D., De Filippis, F., 2024. Microbiome mapping in beef processing reveals safety-relevant variations in microbial diversity and genomic features. *Food Research International* 186, 114318. <https://doi.org/10.1016/j.foodres.2024.114318>

57. Stokes, K., Bennett, N., Fady, P.-E., 2024. Guarding Against Antimicrobial Resistance: Towards Responsible Biocide Use in Consumer Goods. <https://doi.org/10.5281/zenodo.13627461>
58. Stošić, M., Čučak, D., Kovačević, S., Perović, M., Radonić, J., Sekulić, M.T., Miloradov, M.V., Radnović, D., 2016. Meat industry wastewater: microbiological quality and antimicrobial susceptibility of *E. coli* and *Salmonella* sp. isolates, case study in Vojvodina, Serbia. *Water Science and Technology* 73, 2509–2517. <https://doi.org/10.2166/wst.2016.113>
59. Szoke, Z., Fauszt, P., Mikolas, M., David, P., Szilagyi-Tolnai, E., Pesti-Asboth, G., Homoki, J.R., Kovacs-Forgacs, I., Gal, F., Stundl, L., Czegledi, L., Stigel, A., Biro, S., Remenyik, J., Paholcsek, M., 2025. Comprehensive analysis of antimicrobial resistance dynamics among broiler and duck intensive production systems. *Sci Rep* 15, 4673. <https://doi.org/10.1038/s41598-025-89432-z>
60. Tahir, K., Nazir, S., Ahmad, A., Li, B., Khan, A.U., Khan, Z.U.H., Khan, F.U., Khan, Q.U., Khan, A., Rahman, A.U., 2017. Facile and green synthesis of phytochemicals capped platinum nanoparticles and in vitro their superior antibacterial activity. *Journal of Photochemistry and Photobiology B: Biology* 166, 246–251. <https://doi.org/10.1016/j.jphotobiol.2016.12.016>
61. Temple, D., Manteca, X., 2020. Animal Welfare in Extensive Production Systems Is Still an Area of Concern. *Front. Sustain. Food Syst.* 4. <https://doi.org/10.3389/fsufs.2020.545902>
62. Tiedje, J.M., Wang, F., Manaia, C.M., Virta, M., Sheng, H., Ma, L., Zhang, T., Topp, E., 2019. Antibiotic Resistance Genes in the Human-Impacted Environment: A One Health Perspective. *Pedosphere* 29, 273–282. [https://doi.org/10.1016/S1002-0160\(18\)60062-1](https://doi.org/10.1016/S1002-0160(18)60062-1)
63. Valentino, V., De Filippis, F., Sequino, G., Ercolini, D., 2023. Psychrotrophic Bacteria Equipped with Virulence and Colonization Traits Populate the Ice Cream Manufacturing Environment. *Applied and Environmental Microbiology* 89, e00765-23. <https://doi.org/10.1128/aem.00765-23>
64. Valentino, V., Sequino, G., Cobo-Díaz, J.F., Álvarez-Ordóñez, A., De Filippis, F., Ercolini, D., 2022. Evidence of virulence and antibiotic resistance genes from the microbiome mapping in minimally processed vegetables producing facilities. *Food Research International* 162, 112202. <https://doi.org/10.1016/j.foodres.2022.112202>

65. Van Boeckel, T.P., Pires, J., Silvester, R., Zhao, C., Song, J., Criscuolo, N.G., Gilbert, M., Bonhoeffer, S., Laxminarayan, R., 2019. Global trends in antimicrobial resistance in animals in low- and middle-income countries. *Science* 365, eaaw1944. <https://doi.org/10.1126/science.aaw1944>
66. Vieira, T.R., Cavinatto, E.F., Cibulski, S.P., Silva, N.M.V. da, Borba, M.R., Oliveira, C.J.B. de, Cardoso, M.R. de I., 2023. Comparative resistome, mobilome, and microbial composition of retail chicken originated from conventional, organic, and antibiotic-free production systems. *Poultry Science* 102, 103002. <https://doi.org/10.1016/j.psj.2023.103002>
67. Vikram, A., Miller, E., Arthur, T.M., Bosilevac, J.M., Wheeler, T.L., Schmidt, J.W., 2018. Similar Levels of Antimicrobial Resistance in U.S. Food Service Ground Beef Products with and without a “Raised without Antibiotics” Claim. *Journal of Food Protection* 81, 2007–2018. <https://doi.org/10.4315/0362-028X.JFP-18-299>
68. Vikram, A., Rovira, P., Agga, G.E., Arthur, T.M., Bosilevac, J.M., Wheeler, T.L., Morley, P.S., Belk, K.E., Schmidt, J.W., 2017. Impact of “Raised without Antibiotics” Beef Cattle Production Practices on Occurrences of Antimicrobial Resistance. *Applied and Environmental Microbiology* 83, e01682-17. <https://doi.org/10.1128/AEM.01682-17>
69. WHO, 2023. Antimicrobial resistance [WWW Document]. URL <https://www.who.int/news-room/fact-sheets/detail/antimicrobial-resistance> (accessed 2.15.25).
70. Xiao, X., He, M., Ma, L., Lv, W., Huang, K., Yang, H., Li, Y., Zou, L., Xiao, Y., Wang, W., 2024. Insights into microbial contamination and antibiotic resistome traits in pork wholesale market: An evaluation of the disinfection effect of sodium hypochlorite. *Journal of Hazardous Materials* 468, 133811. <https://doi.org/10.1016/j.jhazmat.2024.133811>

CHAPTER 2

Next-Generation Detection of Microbial Spoilage in Meat Supply Chains: Integrating Microbial Dynamics, Metagenomics, and AI-Based Smart Biosensors

Highlights:

- Traditional culture-based methods are inadequate for spoilage detection because they fail to capture the full, complex microbial community.
- Shotgun metagenomics offers deep functional insights but remains too slow and costly for routine industrial adoption.
- The future of spoilage control lies in hybrid systems that combine the genomic depth of multi-omics with the speed of real-time AI and IoT-based biosensors.
- Bridging the "lab-to-industry" gap requires standardized protocols, curated spoilage-gene databases, and supportive policies for new technology.

Abstract:

Meat is a nutrient-dense component of global diets, yet its susceptibility to microbial spoilage presents a significant challenge to food safety and sustainability. This undermines the efforts to meet the rising global demand for animal-based proteins for an ever-growing population. Microbial spoilage primarily driven by *Pseudomonas*, *Brochothrix*, *Clostridium*, and related genera, leads to textural degradation, slime formation, unpleasant odours, and potential health risks. These issues contribute to trillion dollars in global food waste annually. Conventional culture-based detection methods often fail to capture the complexity of spoilage microbiota. In contrast, metagenomics provides a robust, culture-independent approach to detect specific spoilage organisms (SSOs), assess their functional roles, and model spoilage dynamics. However, several barriers limit its industrial application, including host DNA contamination, inconsistent bioinformatics pipelines, lack of meat-specific protocols, and high operational costs that hinder its real-time implementation. Meanwhile, emerging alternatives such as volatile organic compound (VOC) sensors and Internet of Things (IoT)-based platforms, offer rapid, on-site detection but lack the functional resolution of metagenomics. This review explores key developments in the field of meat spoilage from the past two decades and addresses a central question. How can next-generation tools bridge the gap between lab-scale insights and industrial-scale meat spoilage management? It critically evaluates the spoiler's dynamics and the interplay of metagenomics, meta-transcriptomics, and smart biosensors, proposing a roadmap for scalable, multi-omics integration. By linking microbial dynamics to spoilage outcomes across supply chains, this article proposes actionable strategies to reduce food waste, improve safety, and contribute to achieving UN Sustainable Development Goal of 50% food waste reduction by 2030.

Keywords: Meat spoilage; Food safety; Microbial dynamics; Metagenomics; Spoilage detection technologies.

1. Introduction:

Meat is a central component of diets across the globe, valued for its high-quality protein and essential micronutrients such as iron, zinc, vitamins B₁₂ and D, which are vital for immune function and human development. Global meat consumption encompasses poultry (e.g., chicken, turkey, duck), fish, and mammalian meats such as beef, pork, lamb, and buffalo with some exotic meat types as represented in detail. Since 1961, meat production has increased nearly fivefold, marking an exponential growth in the industry (Geiker et al., 2021). Between 1960 and 2010, global meat distribution rose by 204% (Lupton et al., 2017), while meat consumption surged 500% from 1992 to 2016 (Katare et al., 2020). And this demand is expected to increase by 2% from the 2020-2022 base period to 2032 (OECD and FAO, 2023). According to the Food and Agriculture Organization (FAO), global meat production in 2014 reached approximately 318.7 million tons (carcass weight equivalent). On average, each person consumed about 43.5 kilograms (kg) of meat that year. Among the different types of meat, pork was the most consumed at 15.3 kg per person, followed by poultry at 13.8 kg, bovine meat at 10.1 kg, and sheep and goat meat at 2.1 kg per capita (FAO, 2022). As global demand surges, the issue of meat spoilage becomes ever more pressing, carrying significant economic, environmental, and health implications.

Due to its nutrient-rich composition and high water activity, meat is highly perishable and prone to microbial spoilage almost immediately after slaughter, leading to significant food losses (Visciano, 2023). For example, about 25% of the total beef volume is lost throughout the supply chain in countries like Australia, Canada, United States (U.S.), and New Zealand. In the U.S. alone, nearly 194.70 million kilograms of beef are discarded each year, equivalent to losing about 780,000 cattle (Ramanathan et al., 2022). It is estimated that approximately 23% of meat production is lost or wasted globally. The majority of this waste occurs at the consumer level, accounting for 64% of the total, followed by losses during manufacturing (20%), distribution (12%), and primary production and post-slaughter stages (3.5%) (Karwowska et al., 2021). This also leads to a major waste of vital resources like water, animal feed, and energy used in raising livestock. In addition, livestock rearing plays a key role in climate change. About 17 million metric tons of CO₂-equivalent emissions each year come from animal-based foods like beef, due to livestock farming, feed cultivation, and decomposition of wasted meat (Poore and Nemecek, 2018).

Beyond economic and environmental complications, spoiled meat poses serious public health threats. Spoilage organisms like *Pseudomonas* degrade sensory quality, while pathogens such as *Salmonella* and *Listeria monocytogenes* can cause life-threatening foodborne illnesses (USDA, 2025; Zhang et al., 2025). In 2010, the World Health Organization (WHO) reported 600 million foodborne illness cases and 420,000 related deaths (WHO, 2015). Moreover, key pathogens including *Salmonella*, *L. monocytogenes*, *E. coli* O157, *Campylobacter*, and *Toxoplasma* are responsible for about 33,000 hospitalizations, 3.5 million infections, and 1,600 deaths annually (Lee et al., 2021). Additional concerns include *Clostridium botulinum*, the agent of botulism, and *Yersinia enterocolitica*, often linked to vacuum-packed and undercooked pork (Parmley et al., 2013; Sobel and Lúquez, 2021). Recent evidence also shows that the decomposition of meat waste in landfills contributes to the airborne spread of pathogens and antibiotic resistance genes, highlighting an overlooked pathway of public and environmental health risk (Yu et al., 2021). **Figure. 1** illustrates the economic, environmental, and health consequences of meat spoilage.

Despite the urgency, current detection methods have significant limitations. Traditional culture-based techniques often fail to capture the full microbial diversity responsible for spoilage. For instance, *Clostridium* species in a viable but non-culturable (VBNC) state can evade detection, yet they play a dominant role in vacuum-packed meat spoilage (Indio et al., 2024). Advanced approaches like metagenomics also face challenges, including inconsistent DNA extraction, host DNA interference, and lack of harmonisation and standardization in bioinformatics tools, all of which hinder reproducibility (Grützke et al., 2019; Sabater et al., 2021). Additionally, the presence of non-spoilage microbes and environmental contaminants can lead to false positives or negatives, especially at the species level, and DNA from dead bacteria further complicates interpretation (Grützke et al., 2019; Srinivas et al., 2022). These methods require expensive infrastructure and skilled personnel in computational biology and bioinformatics, making large-scale adoption unfeasible (Fletcher et al., 2018; Sabater et al., 2021). Moreover, sequencing, and downstream analysis are time-intensive, often requiring days or weeks, rendering real-time decision-making in meat monitoring impractical (Binson and Thomas, 2023; Xie et al., 2016).

To address the need for timely decisions and scalability in the meat industry and retail markets, VOC-based and AI-based monitoring systems have been developed for rapid, on-site detection of meat spoilage. However, these approaches have their own limitations. VOC sensors can

suffer from sensors limited selectivity and specificity in complex food matrices, as they may respond to a mixture of gases, making it challenging to pinpoint specific spoilage organisms. Additionally, sensor drift over time can affect accuracy, and environmental factors like humidity and temperature can influence sensor responses, leading to potential false readings (Damdam et al., 2023; Lawaniya et al., 2025). Similarly, AI-based systems, while promising, require extensive training datasets to accurately predict spoilage, and their performance can be hindered by the variability of meat matrices and storage conditions (Damdam et al., 2023; S. Kolbeck et al., 2021).

Given these challenges, there is a pressing need for a comprehensive review that critically evaluates the microbial spoilage dynamics, current detection methods and explores emerging technologies in meat spoilage monitoring. This review seeks to address a critical challenge: how can emerging technologies be optimized to overcome these limitations and offer scalable, fast, real-time solutions for managing meat spoilage? This review article explores the integration of metagenomics, meta-transcriptomics, and IoT-enabled VOC sensors, aiming to connect microbial behaviour with spoilage outcomes across the entire meat supply chain. By proposing a roadmap for multi-omics integration with AI-based sensors, this study aims to promote waste reduction, improve food safety, and contribute to global efforts, such as UN Sustainable Development Goals (SDG 12.3) to cut food loss by half by 2030.

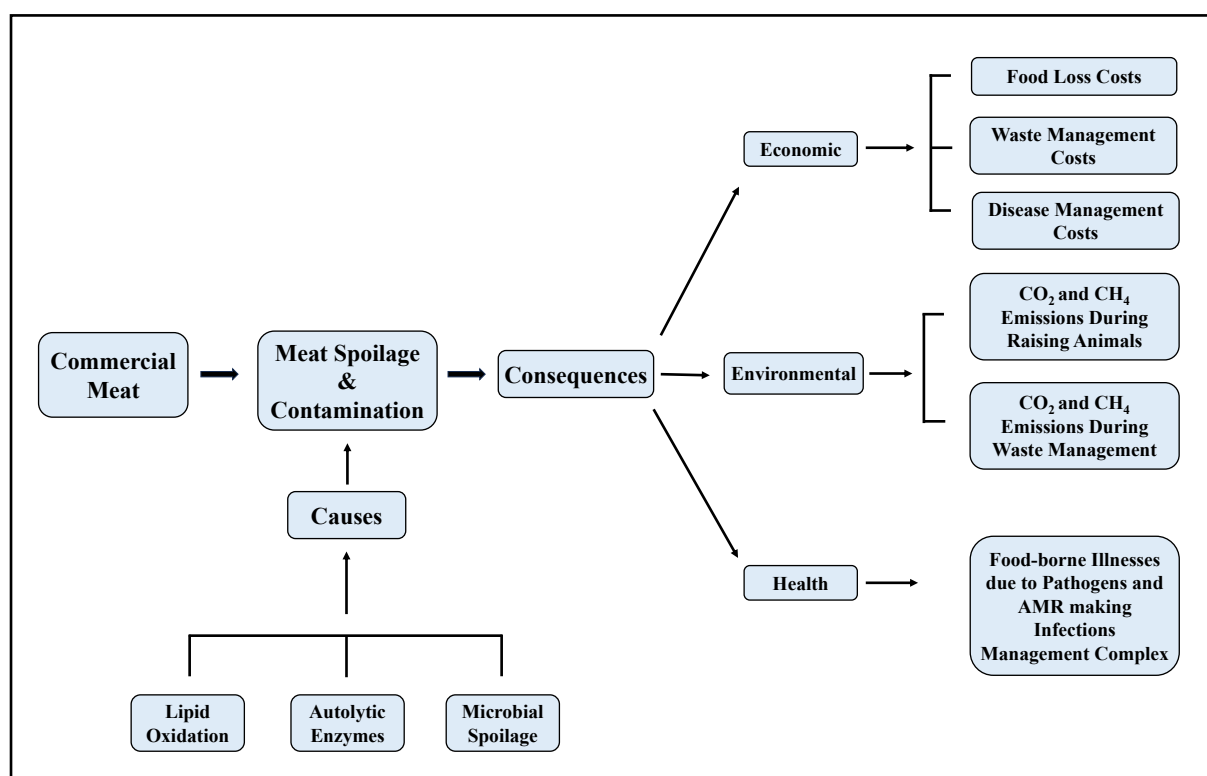


Figure: 1. This schematic presentation summarizes the causes of meat spoilage and its consequences in terms of economic burden, environmental impacts and health issues related to meat spoilage.

2. FACTORS CONTRIBUTING TO MEAT SPOILAGE

Meat spoilage is influenced by a complex interplay of intrinsic and extrinsic factors that affect microbial growth, enzymatic activity, and chemical degradation. Both intrinsic and extrinsic factors play a significant role in determining spoilage outcomes (Patarata et al., 2020).

2.1. Intrinsic factors

2.1.1. Meat Composition:

Meat is a rich source of proteins, peptides, amino acids, and lipids, all of which support the growth of diverse microbial populations. The types of microbial consortia that develop on meat are directly influenced by the nutrients available for growth. Growth factors and inhibitory compounds collectively shape microbial diversity. Spoilage bacteria utilize these substrates, producing Volatile Organic Compounds (VOCs) leading to off odours. The balance between different nutrient types can influence the dominant spoilage flora; for instance, protein-rich

substrates favour proteolytic bacteria, while lipid-rich environments promote lipid-oxidizing organisms (Silva et al., 2021; Pellissery et al., 2020).

2.1.2. pH:

In addition to meat composition, pH plays a crucial role in spoilage dynamics. At slaughter, meat typically has a pH of around 7.0, but it drops to 5.5–6.2 within a few hours post-mortem. Lower pH levels generally inhibit the growth of spoilage microorganisms, particularly gram-negative bacteria, while favoring lactic acid bacteria (LAB). Higher pH, particularly in adipose tissues, may encourage the proliferation of other bacterial taxa. A notable example is Dark Firm Dry (DFD) meat, which results from pre-slaughter stress and exhibits a pH range of 6.2–6.5. This may create a more favourable environment for certain spoilage bacteria, allowing them to proliferate faster and degrade the meat (Ahmad Mir et al., 2017; Hwang et al., 2020).

2.1.3. Water Activity:

Another critical intrinsic factor is water activity (a_w). Fresh beef has an a_w of approximately 0.99, while chilled meat ranges between 0.96–0.97. High a_w levels provide an ideal setting for microbial proliferation on meat surfaces, which accelerates spoilage rates (Odeyemi et al., 2020). Reduction in a_w through methods like drying, salting, or freezing can significantly delay spoilage. However, many psychrotrophic spoilage organisms, such as *Pseudomonas* spp., remain active at relatively low a_w levels, particularly under refrigerated, aerobic conditions (Odeyemi et al., 2020).

2.2. Extrinsic factors

2.2.1. Temperature:

Among the external contributors, temperature is perhaps the most pivotal. Different microorganisms have distinct temperature preferences, and without adequate chilling, spoilage can escalate rapidly. To counteract this, strict temperature control is necessary. According to EU regulations, post-mortem carcasses should be cooled to $\leq 7^\circ\text{C}$ and viscera to $\leq 3^\circ\text{C}$ to preserve meat quality (EU Commission Regulation (EC) No 853/2004, Annex III, Section I, Chapter VII, point 1(a)). However, psychrotrophic bacteria like *Pseudomonas* spp. and *Brochothrix thermosphacta* can still proliferate at $0\text{--}4^\circ\text{C}$, leading to spoilage. Fluctuations or interruptions in the cold chain significantly accelerate deterioration (Luong et al., 2020).

2.2.2. Packaging:

In addition to temperature, the packaging conditions significantly influence microbial growth. Packaging type, whether vacuum-sealed, modified atmosphere, or aerobic directly influences spoilage dynamics. For instance, Kolbeck et al. (2021) and Wickramasinghe et al. (2021) observed that *Pseudomonas* spp. and *Enterobacteriaceae* produce sulphurous and cheesy odours during aerobic storage (1–25°C), contributing to spoilage (Sandra Kolbeck et al., 2021b; Wickramasinghe et al., 2021). Modified Atmosphere Packaging (MAP), particularly with high O₂ levels, promotes the growth of *Brochothrix* spp. and *Serratia* spp., the latter being a dominant genus among *Enterobacteriaceae* in MAP conditions (Carrizosa et al., 2017; Casaburi et al., 2014). In contrast, vacuum packaging reduces oxygen levels, which inhibits aerobic bacteria like *Pseudomonas*, but can encourage the growth of facultatively anaerobic microbes such as lactic acid bacteria, and anaerobic *Clostridium* spp. (Carrizosa et al., 2017; Zhang et al., 2018). The factors responsible for meat spoilage and specific microbial groups (in terms of their physiology and metabolic potential) are summarized in **Figure 2**.

2.2.3. Relative Humidity and Light Exposure:

High humidity can support microbial growth, especially on meat surfaces. Light exposure, especially UV light, may accelerate lipid oxidation, resulting in rancid odors (Amaral et al., 2018). However, light can also suppress surface microbial growth if used appropriately (e.g., UV-C interventions), though this requires careful control to avoid oxidative quality loss (Wang et al., 2023).

2.2.4. Processing, Handling and Hygienic Practices:

The level of hygiene during slaughter, processing, and storage plays the most fundamental role in spoilage onset. Cross-contamination from tools, surfaces, or personnel can introduce spoilage or pathogenic microbes. The cleanliness of chillers, cutting boards, conveyor belts, and other contact surfaces significantly affects the microbial loads and spoilage trajectory of meat. Similarly, frequent handling during processing or distribution increases the risk of microbial contamination and structural damage, accelerating deterioration. Lastly, the unsterile packaging at the end of the meat supply chain further introduces microbial contaminants (Cobo-Díaz et al., 2021; De Filippis et al., 2021; Valentino et al., 2022; Sequino et al., 2024; Barcenilla et al., 2024; De Filippis et al., 2024; Valentino et al., 2023).

3. TYPES OF MEAT SPOILAGE

After slaughter, during processing, and while being stored, meat spoilage manifests through three interconnected pathways: (a) lipid oxidation, (b) autolytic enzymatic activity, and (c) microbial degradation. These spoilage mechanisms not only compromise sensory quality but also pose food safety risks and drive economic losses. Understanding their interplay is essential for designing effective preservation strategies across the meat supply chain.

3.1. Lipid Oxidation:

Lipid oxidation is a major chemical reaction that leads to meat spoilage, resulting in sensory deterioration, nutrient loss, and potential health hazards. Although precise percentages of spoilage attributed solely to lipid oxidation are not uniformly quantified, research consistently cites it as a leading cause of chemical spoilage. For example, fat rich in polyunsaturated fatty acids (PUFAs), is highly susceptible to oxidation and it contributes significantly to rancidity and to the development of off flavours in meat products (Amaral et al., 2018). Lipid oxidation follows the autoxidation pathway, a process where reactive oxygen species attack PUFAs, leading to the formation of peroxides, aldehydes, and ketones. Vacuum packaging may help reducing oxidation by limiting oxygen exposure. In fact, studies have reported up to 50% lower TBARS (thiobarbituric acid reactive substances) in vacuum-packaged cooked meat compared to air-exposed samples (Papuc et al., 2017). Similarly, chemical preservatives like Tert-butyl hydroquinone (TBHQ) and ascorbic acid help reducing oxidative spoilage (Oduoye et al., 2024).

3.2. Autolytic Enzymatic Spoilage:

While lipid oxidation primarily affects the chemical composition of the meat, autolytic enzymatic spoilage plays a crucial role in texture degradation. Autolytic enzymatic spoilage is a natural post-mortem process within muscle cells: enzymes break down muscle proteins, often providing molecules that serve as precursors to microbial degradation, significantly altering meat texture, appearance, and quality (Dave and Ghaly, 2011; Luong et al., 2020; Nychas et al., 2008; Oduoye et al., 2024).

3.3. Microbial Spoilage:

Among the three pathways, microbial spoilage has the greatest significance in the meat industry due to its direct impact on economic losses and food waste. Meat is an excellent substrate for microbial proliferation. Spoilage microbes degrade meat's chemical components, producing

by-products that alter its sensory characteristics e.g., colour, odour, texture, and flavour, ultimately rendering it unacceptable.

The main microbial culprits include bacteria, archaea, yeasts, and molds. For example, *Pseudomonas* spp. are common spoilage organisms in poultry, while *Clostridium* spp. often dominate in vacuum-packed meats like beef (Zhang et al., 2018). Similarly, other bacterial taxa associated with spoilage under refrigeration include *Carnobacterium*, *Enterobacteriaceae*, *Lactobacillus*, *Leuconostoc*, *Brochothrix thermosphacta*, *Acinetobacter*, *Flavobacterium*, *Psychrobacter*, and *Moraxella* species (Fletcher et al., 2018; Hwang et al., 2020). Although meat is largely sterile at slaughter, contamination begins during processing due to exposure to external processing environments.

Microbial growth depends on intrinsic factors (water activity, nutrient composition, pH) and extrinsic factors (temperature, oxygen availability, packaging). For instance, vacuum packaging reduces aerobic spoilage organisms like *Pseudomonas*, but may favor anaerobic or facultative anaerobic bacteria like lactic acid bacteria, which can still produce spoilage metabolites under reduced oxygen, continuing the deterioration process (Hwang et al., 2020; Zhu et al., 2022a).

Different spoilage microbes exhibit distinct behaviours. *Pseudomonas* and *Shewanella putrefaciens* produce sulfur-containing compounds like hydrogen sulfide, creating foul odours. Lactic acid bacteria like *Lactobacillus* and *Leuconostoc* ferment sugars into organic acids, causing sour flavours and even greening of meat through oxidative by-products. Microbial activity can also decrease pH or alter colour via pigment production or oxidative reactions (Borch et al., 1996).

Microbial growth and activity vary depending on storage conditions. *Pseudomonas* spp., for example, are psychrotrophs that thrive in cold, oxygen-rich environments, often dominating aerobic refrigerated meat. These bacteria secrete extracellular enzymes that degrade proteins and fats, causing spoilage symptoms such as slime formation, off-odours, and discoloration (Hwang et al., 2020; Zhu et al., 2022a).

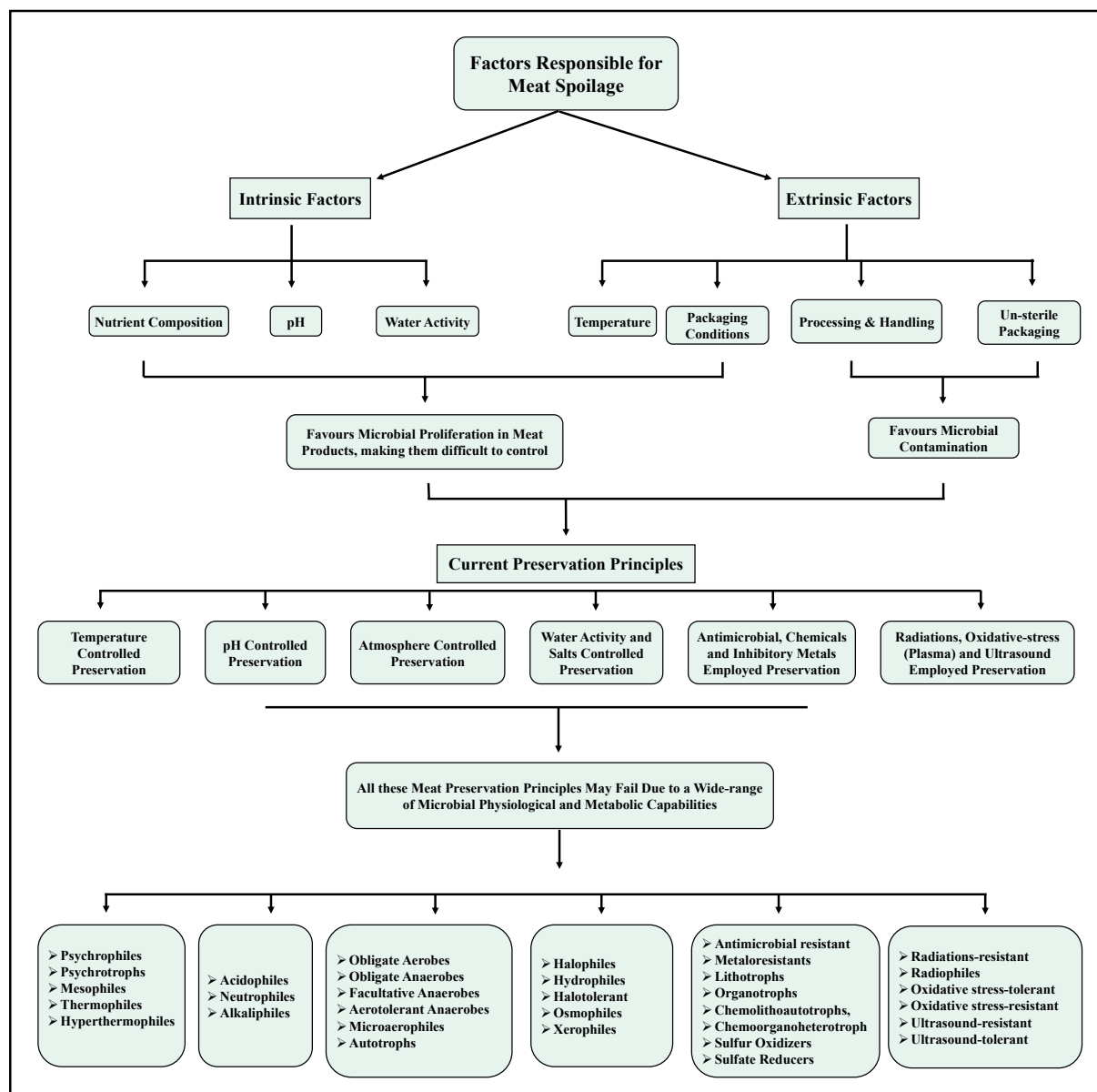


Figure 2: Schematic summarizing factors driving meat spoilage. Extrinsic factors (e.g., processing, handling, un-sterile packaging) introduce microbial contaminants, while some extrinsic factors (temperature and packaging conditions) and intrinsic factors (e.g., pH, aw, and nutrients) enable selective proliferation of spoilage-associated microbial taxa. Although diverse microbes colonize meat, spoilage is primarily mediated by organisms capable of metabolizing meat components (e.g., proteins, lipids). **Complete decontamination is unattainable; even if only one or a few microbes remain, these can proliferate and lead to spoilage, causing major economic losses.** Thus, reducing initial microbial loads remains critical. Preservation strategies (e.g., vacuum packaging, chilling) target specific physiological traits but face limitations due to microbial metabolic diversity, encompassing multiple

physiological groups. For example, vacuum packaging inhibits aerobes but favours anaerobes like *Clostridium*, while chilling selects for psychrotrophs such as *Pseudomonas*. Which makes the application of preservation methods based on single physiologic principles unworthy. Therefore, hurdle methods (e.g., combining acidity, salinity, and temperature) disrupting multiple microbial pathways are more capable of extending shelf life. However, adaptability of certain taxa may eventually lead to spoilage, highlighting the need for dynamic preservation strategies.

Meat processing, from farm to consumer, involves multiple stages that influence the microbiome of meat, affecting its safety, quality, and characteristics. The microbiome of livestock is shaped by several factors, including diet, genetics, immune health, antibiotic administration, and environmental conditions, with farming practices playing a key role in the development of microbial populations (Van Gompel et al., 2020). The co-existence of the livestock and human gut microbiome contributes to the composition of the faecal microbiome and the resistome, which can transfer antimicrobial resistance genes to spoilage organisms, complicating meat preservation and posing risks to public health. The microbiome development in poultry, from pre-hatching stages, also impacts their health and growth, affecting resistance to microbial infections (Van Gompel et al., 2020).

SSOs actively degrade meat by metabolizing substrates that lead to odour changes, texture modifications, and colour variations (Fu et al., 2018). SSOs are defined by their dominance in spoilage consortia and metabolite production (Gram and Dalgaard, 2002). In terms of specific meat types, livestock meat from cattle, pigs, lamb, and sheep is especially prone to spoilage due to SSOs, which break down proteins and fats, resulting in undesirable odours, sliminess, and structural changes in the meat (Zhao et al., 2022). Poultry, including chicken, turkey, and duck, is highly susceptible to spoilage even under refrigerated conditions, with diverse microbial communities, including both spoilage and pathogenic microorganisms, contributing to its degradation (Dave and Ghaly, 2011; Pellissery et al., 2020). Fish, due to its high moisture content and pH, is similarly vulnerable to microbial spoilage, with SSOs being the primary culprits in the spoilage process, similar to other meat types (Gram and Dalgaard, 2002). These spoilage patterns are further detailed in **Table 1**, which provides a comprehensive overview of the microorganisms involved in spoilage across different types of meat.

Microbial spoilage can be caused by various microorganisms, including bacteria, yeasts, and molds, which can thrive in both aerobic (with oxygen) and anaerobic (without oxygen) conditions (Pellissery et al., 2020). In aerobic conditions, spoilage is often marked by surface changes like sliminess, discoloration, rancidity, and pigmentation, with bacteria such as *Pseudomonas*, *Acinetobacter*, *Brochothrix* and *Bacillus* playing a significant role. Pigmentation can also be caused by specific species like *Serratia marcescens* (red spots) and *Pseudomonas synchyanea* (blue) (Rao et al., 2021). Fungal contaminants like *Mucor* and *Penicillium* can create black, green, or white spots, while yeasts cause fat degradation and off-odours (Rao et al., 2021). In anaerobic conditions, spoilage is typically characterized by putrefaction, with bacteria like *Clostridium* and *Proteus* producing foul-smelling compounds as they break down proteins (Sobel and Lúquez, 2021). Additionally, lactic acid bacteria can cause souring by fermenting sugars, lowering the pH, and producing sour odours and flavours (Dave and Ghaly, 2011). These multi-group spoilers are further detailed in **Table 2**, which outlines the main microbial taxa involved in spoilage across different meat types.

Microorganisms such as bacteria and fungi employ complex biochemical processes that break down meat components into undesirable compounds. One major spoilage pathway is putrefaction, which occurs under anaerobic conditions. Bacteria such as *Vibrio*, *Alcaligenes*, and *Spirillum* degrade proteins and produce hydrogen sulfide (H_2S), which causes the characteristic rotten egg smell (Dave and Ghaly, 2011; Zhu et al., 2024). Additionally, lactic acid bacteria (LAB) like *Lactobacillus* and *Leuconostoc* can cause greening discoloration by producing hydrogen peroxide (H_2O_2), while nitrate-reducing bacteria release nitric oxide gas, which can alter meat colour (Hugas et al., 2003; Sandra Kolbeck et al., 2021b). Other bacteria, such as *Pseudomonas* and *Bacillus*, produce volatile compounds like trimethylamine (TMA) and sulfur compounds, which contribute to off-flavours, while *Streptomyces* contributes to proteolysis (Cervený et al., 2009; Sandra Kolbeck et al., 2021a). Furthermore, bacteria produce fermentation products, such as acetate and propionate, which cause foul odours and discoloration (Cervený et al., 2009). Additionally, LAB and *Brochothrix* spp. produce acids, gases, and slime, which degrade the meat's texture and contribute to its unpleasant odour (Casaburi et al., 2014; González-Fandos et al., 2025). For more details on the specific mechanisms employed by various microbes, refer to **Table 3**.

Table 1: Spoilage Microorganisms in Different Types of Meat

Meat type	Dominant spoilage microorganisms	Storage conditions & effects	References
Livestock Meat	<i>Klebsiella, Escherichia, Proteobacteria, Firmicutes, Pseudomonas, Acinetobacter, Pantoea, Brochothrix, Raoultella</i>	Found in meatballs with blown pack spoilage (BPS) under 71.85% CO ₂ ; Packaging swelling; Spoilage in chilled pork at -2°C and 4°C; decomposition of proteins and fats leads to off-odors and textural changes.	(Chen et al., 2022; X. Wang et al., 2021; Zhao et al., 2022)
	<i>Acinetobacter, Myroides, Kurthia, Pseudomonas and Brochothrix.</i>	Markers of spoiled pork at 25°C.	(Zhao et al., 2022)
	<i>Clostridium sensu stricto_2</i> (92.01%), <i>Aspergillus</i> (84.27%), <i>Micrococcaceae</i> ,	Identified in dry-cured hams (Mianning ham); affected by moisture and salt levels.	(Liao et al., 2022; Zhu et al., 2022b)
	<i>Enterobacteriaceae, Enterococcus</i>	Found in high moisture, low-salt conditions in ham.	(Zhou et al., 2022, 2021)
	<i>C. farmeri, B. cereus, E. faecalis</i>	Identified in Jinhua ham spoilage.	(Zhan et al., 2021)

	<i>Clostridium estertheticum</i>	Causes BPS in vacuum-packaged beef.	(Yang et al., 2014)
	<i>Lactobacillus, Enterobacteriaceae, Pseudomonas.</i>	Found in dry-aged beef, affecting texture and aroma.	(Li et al., 2014)
	<i>Psychrophilic Clostridium.</i>	Major spoilage contributors in beef and lamb, particularly in colder climates.	(Wambui et al., 2021)
Poultry Meat	<i>Pseudomonas, Bacillus, Salmonella, Aeromonas, Schwartzella.</i>	Dominate in poultry stored under aerobic conditions.	(Odeyemi et al., 2020; Wang et al., 2017)
	<i>Clostridium perfringens, Pseudomonas fluorescens, Aeromonas salmonicida, Serratia liquefaciens</i>	Cause rapid spoilage in poultry at 8°C within 4 days.	(Wang et al., 2017)
	<i>Viikkiensis enterococcus, Seigonensis enterococcus</i>	Newly identified <i>enterococci</i> affecting poultry meat spoilage.	(Harada et al., 2016; Rahkila et al., 2011)
	<i>Pseudomonas fragi, P. lundensis, P. fluorescens, P. aeruginosa</i>	Produce slime and soften poultry meat by breaking down myogenic proteins.	(Katiyo et al., 2020; Rouger et al., 2017)
	<i>Serratia, Micrococcus, Brucella</i>	Associated with slime production and softening of poultry meat.	(Pellissery et al., 2020)

Fish Meat	<i>Pseudomonas, Salmonella, Shewanella</i>	Significant contributors to fish meat spoilage under aerobic and modified atmosphere conditions.	(Møretrø et al., 2016; Zhang et al., 2015)
	<i>Karnococcus, Pseudomonas aeruginosa.</i>	Predominant in vacuum-packaged and air-packaged carp.	(Zhang et al., 2015; Zhu et al., 2022a)
	<i>Photobacterium</i>	CO ₂ -resistant bacteria found in fish stored under modified atmospheric conditions.	(Murhekar et al., 2017)
	<i>Shewanella</i>	Produces biogenic amines in raw tuna and swordfish, causing strong odors.	(SERIO et al., 2011)
	<i>Brochothrix thermosphacta</i>	Produces off-flavors in seafood stored aerobically.	(Macé et al., 2012)
	<i>Psychrobacter spp. (Psb. immobilis)</i>	Breaks down lipids and amino acids, leading to musty odors in seafood.	(Broekaert et al., 2013)

Table 1: Main Microbial Taxa Involved in Different Types of Meat Spoilage

Microbial Taxa	Family	Meat type	References
<i>Geotrichum, Cladosporium, Penicillium, Sporotrichum, Mucor</i>	Various (Molds)	All meat types	(Fletcher et al., 2018)
<i>Candida, Rhodotorula, Cryptococcus</i>	Various (Yeasts)	All meat types	(Fletcher et al., 2018)
<i>Clostridium estertheticum, Cl. gasigenes</i>	Clostridiaceae	Vacuum-packed meat (gas-producing)	(André et al., 2017; Palevich et al., 2021)
<i>Cl. aligidicarnis, Cl. bowmanii, Cl. frigoris, Cl. frigidicarnis</i>	Clostridiaceae	Vacuum-packed meat (non-gas)	(André et al., 2017)

<i>Pseudomonas fragi</i> , <i>P. fluorescens</i> , <i>P. lundensis</i>	Pseudomonadaceae	Chilled, aerobically stored meat	(Stellato et al., 2017)
<i>Shewanella putrefaciens</i>	Shewanellaceae	Chilled, vacuum-packed beef	(Gladding and Gwyther, 2017)
<i>Serratia liquefaciens</i> , <i>Hafnia alvei</i> , <i>Pantoea agglomerans</i>	Enterobacteriaceae	Fresh meat (temperature abuse)	(G. Wang et al., 2021)
<i>Lactic acid bacteria (LAB)</i> , <i>Brochothrix thermosphacta</i>	Lactobacillaceae, Listeriaceae	Cold, aerobically stored meats	(Raposo et al., 2016)
<i>Clostridium</i> , <i>Alcaligenes</i> , <i>Proteus</i> , and <i>Lactic acid bacteria</i>	Lactobacillaceae, Clostridiaceae, Alcaligenaceae, and Enterobacteriaceae	Anaerobic spoilage	(Dave and Ghaly, 2011)
<i>Acinetobacter</i> , <i>Arthrobacter</i> , <i>Achromobacter</i> , <i>Aeromonas</i> , <i>Alcaligenes</i> , <i>Leuconostoc</i> , <i>Citrobacter</i> , <i>Alteromonas</i> , <i>Paenibacillus</i> , <i>Microbacterium</i> , <i>Campylobacter</i> , <i>Bacillus</i> , <i>Escherichia</i> , <i>Kocuria</i> , <i>Lactococcus</i> , <i>Carnobacterium</i> , <i>Corynebacterium</i> , <i>Flavobacterium</i> , <i>Klebsiella</i> , <i>Kluyvera</i> , <i>Kurthia</i> , <i>Lactobacillus</i> , <i>Listeria</i> , <i>Micrococcus</i> , <i>Enterobacter</i> , <i>Moraxella</i> , <i>Streptococcus</i> , <i>Proteus</i> , <i>Providencia</i> ,	Various taxonomic groups	Various meat types	(Mahmud et al., 2023; Rao et al., 2021; Rawat, 2015; Saenz-García et al., 2020)

<i>Staphylococcus, Vibrio,</i> <i>Weissella, Yersinia,</i> <i>Serratia marcescens,</i> <i>Pseudomonas</i> <i>syncyanea,</i> <i>Chromobacterium</i> <i>lividum,</i> <i>Flavobacterium,</i> <i>Thamnidium, Mucor,</i> <i>Cladosporium,</i> <i>Sporotrichum,</i> <i>Penicillium,</i> <i>Photobacterium,</i> <i>Actinomycetes</i>			
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Table 3: Mechanisms Employed by Various Microbes in Meat Spoilage

Micro-organisms	Spoilage Mechanisms and Metabolites	References
<i>Lactobacillus fructivorans, L. viridescens, L. jensenii, Enterococcus faecium, Leuconostoc, Enterococcus faecalis, Pediococcus</i>	Greening with H ₂ O ₂	(Dave and Ghaly, 2011; Hugas et al., 2003; Sandra Kolbeck et al., 2021a, 2021b)
Nitrate-reducing bacteria	Nitric oxide gas production	(Kim et al., 2022)
Micrococcus and yeasts	Slime layer on the surface	(Cervený et al., 2009)
<i>Vibrio, Alcaligenes, Spirillum, Enterobacteriaceae</i>	Putrefaction, H ₂ S production	(Cervený et al., 2009; Dave and Ghaly, 2011; Hugas et al., 2003)
<i>P. putida, P. fragi, P. fluorescens, P. lundensis,</i>	Proteolysis, Ammonia production and other malodorous compounds	(Cervený et al., 2009; Sandra Kolbeck et al., 2021a)
<i>Acinetobacter, Moraxella, Flavobacterium</i>	Limiting the oxygen availability	(Casaburi et al., 2015)
<i>Brocothrix spp</i>	Acid production (acetic, formic isobutyric, isovaleric)	(Casaburi et al., 2015; González-Fandos et al., 2025)

Lactic Acid Producing Bacteria (LAB)	lactic acid, slime, and carbon dioxide production	(Cervený et al., 2009; Dave and Ghaly, 2011)
<i>Weissella (W. viridescens)</i>	Gas production	(Cervený et al., 2009; Dave and Ghaly, 2011)
<i>Serratia, Proteus, Enterobacter, Hafnia</i>	Metabolize amino acids	(Cervený et al., 2009; Dave and Ghaly, 2011)
<i>Pseudomonas spp. and Carnobacterium</i>	Alcohol production	(Casaburi et al., 2015)
<i>Pseudomonas, Carnobacterium, Enterobacteriaceae</i>	Aldehyde production	(Sandra Kolbeck et al., 2021a)
<i>Carnobacterium, B. thermosphacta, Lactobacillus</i>	Acetoin production	(González-Fandos et al., 2025)
<i>P. fragi</i>	Ester production	(González-Fandos et al., 2025)
<i>B. thermosphacta and Carnobacterium</i>	Volatile acids production	(Casaburi et al., 2015)
<i>Pseudomonads</i>	Sulphur compounds	(Sandra Kolbeck et al., 2021a)
<i>Alcaligenes</i>	Amino acids and N compounds utilization	(Dave and Ghaly, 2011)
<i>Bacillus</i>	Amino acids utilization, trimethylamine (TMA) production, and sulfur compounds	(Cervený et al., 2009)
<i>Staphylococcus</i>	Amino acids, and TMAO utilization	(Dave and Ghaly, 2011)
<i>Streptococcus</i>	Amines and acid production	(Dave and Ghaly, 2011)
<i>Streptomyces</i>	Proteolysis	(Dave and Ghaly, 2011)

4. MICROBIAL DETECTION IN MEAT SPOILAGE: A COMPARATIVE OVERVIEW

Meat quality and safety is crucial for both consumers and the meat industry. While we have methods to detect spoilage-causing microbes, but they are not perfect. Current detection techniques can be slow, expensive, or not sensitive enough. As demand for better quality meat grows, we need faster, more accurate, and affordable solutions. Improved detection methods are key to identifying contamination early and preventing unsafe meat from reaching to consumers. This section explores the methods used to detect microbial spoilage, emphasizing why constant improvements are necessary for food safety and quality control.

4.1. Traditional Culture-Based Methods:

Traditional culture-based methods are commonly used to detect microorganisms in meat. These methods involve isolating bacteria from meat samples using selective media and then growing them in lab to identify and/or characterise isolates. For instance, through culture-based methods it was shown that, in VP-packed minced meat, a mix of *S. carnosus* and *L. sakei* effectively suppressed *Enterobacteriaceae* and *Pseudomonas spp.* (Xu et al., 2023). And Marmon et al. (2023) noted differences in pathogen-associated genera across various meat processors using culture-dependent methods (Marmion et al., 2023). However, this approach has limitations, as it can only identify microorganisms that are culturable, meaning it may miss those that are viable but non-culturable (VBNC) or anaerobic or fastidious microbes.

4.2. PCR-based Methods:

Polymerase Chain Reaction (PCR) and quantitative PCR (qPCR) are molecular techniques that can detect specific DNA sequences from microorganisms. PCR amplifies targeted DNA, while qPCR allows for quantifying it in real-time, making it more sensitive and faster than culture methods. For instance, one study used qPCR to detect and quantify 10 lactic acid bacterial genera in cold-smoked salmon, while another applied multiplex qPCR to identify *Pseudomonas*, *Staphylococcus*, and *Brochothrix thermosphacta* in meat samples, achieving 100% sensitivity and specificity (Bahlinger et al., 2021; Jérôme et al., 2022). Similarly, using multiplex-PCR for *carA* gene, *Pseudomonas spp.* at 10^3 CFU/g in all samples of beef cuts was successfully quantified, regardless of retail type (De Filippis et al., 2018). Likewise, another study demonstrated the reliability of digital droplet PCR (ddPCR) in detecting spoilage, finding no microbiological deterioration or significant increase in histamine and putrescine-producing genes by day 15 in MAP groups (Bayrakal and Çiftçioğlu, 2023). However, PCR-based detection methods can be limited by complex sample preparation, sensitivity to inhibitors, high costs, target-specific detection, potential false positives, and lack of real quantitative data.

4.3. Metabarcoding:

Metabarcoding is another molecular approach that uses specific marker genes, such as 16S rRNA, to profile the diversity of microbial communities in meat. Unlike PCR, metabarcoding can detect a wide range of species, including those that are difficult to culture. For instance, a study using 16S rRNA gene sequencing was able to identify over 600 operational taxonomic

units (OTUs) across 15 different phyla in carcass swabs (Filippis et al., 2013). Similarly, another study revealed more than 800 OTUs at the species level, with core microbiota shared by 80% of the samples, including *Pseudomonas*, *Streptococcus*, *Brochothrix*, *Psychrobacter*, and *Acinetobacter* species (Stellato et al., 2016). However, metabarcoding has its own limitations. It can sometimes be biased by primer selection, meaning certain microorganisms may not be quantified or even detected. Similarly, this technique can only identify the already known microbes.

4.4. Shotgun Metagenomics:

Shotgun metagenomics offers a more comprehensive method for microbial detection. Unlike PCR or metabarcoding, it sequences all DNA present in a sample, allowing for the identification of a wide range of microorganisms and their potential functions. This method provides insights into microbial interactions and metabolic pathways that are involved in spoilage processes, such as amino acid degradation or biogenic amine production. Being a cutting-edge technique, multiple studies have used shotgun sequencing to taxonomically identify, and quantify the relative abundances of the underlying microbiome in meat supply chains. Furthermore, uncovering the metabolic potential, such as spoilage related and antimicrobial resistance genes, of the whole microbiome across the meat supply chain (Cobo-Díaz et al., 2021; De Filippis et al., 2021; Valentino et al., 2022; Sequino et al., 2024; Barcenilla et al., 2024; De Filippis et al., 2024; Valentino et al., 2023). However, shotgun sequencing can be expensive, time consuming, and requires trained personnel. Likewise, data analysis can be complex, and can be hindered by database limitations and host DNA interference. Another major drawback is that it cannot differentiate between the live and dead microbes.

4.5. Meta-transcriptomics:

Meta-transcriptomics goes one step further by analysing RNA to detect which genes are actively being expressed in the microbial community. This approach provides insights into the functional activity of microbes, such as identifying active spoilage pathways. A meta-transcriptomic study found that psychrotrophic lactic acid bacteria (LAB), particularly *Leuconostoc* species, were the dominant active LAB in SSOs. The community responded to environmental stress with cold-shock reactions, followed by the activation of genes to manage oxidative stress (Hultman et al., 2020). Similarly, another meta-transcriptomics study explored

how fermentation by *Pediococcus acidilactici* and *Rhizopus oryzae* affects the quality and odour metabolism in mutton sausages (N. Chen et al., 2025). However, RNA is less stable than DNA, which makes it harder to collect, process and store in real-world conditions.

4.6. Meta-proteomics:

Meta-proteomics involves the analysis of proteins produced by a microbial community, providing direct evidence of microbial metabolic activity. This method allows for the identification of enzymes and proteins involved in spoilage processes. A meta-proteomics study highlighted the key role of microbial glycolytic enzymes in pork spoilage (Gu et al., 2024). Similarly, another study identified species-specific peptide biomarkers to pinpoint bacteria responsible for seafood decay (Abril et al., 2024). However, meta-proteomics faces challenges, including difficulties in protein extraction, and the complexity of identifying all proteins in a sample (e.g., protein annotation limitations).

4.7. Metabolomics:

Metabolomics focuses on identifying and quantifying metabolites produced by microorganisms in meat matrices, primarily using mass spectrometry techniques such as GC-MS and LC-MS. Many modern spoilage detection tools, such as VOC-based biosensors, electronic noses, smart biosensors, and AI-powered sensor platforms. All these methods rely on metabolomics-derived outputs. The portability and scalability of these technologies make them ideal for real-time meat spoilage monitoring. Key rapid detection methods are summarized in **Table 4**. These techniques can detect spoilage-associated VOC compounds like methanethiol and isopropanol, while laser speckle decorrelation allows non-contact monitoring of microbial activity. When integrated with machine learning, these tools enable fast, reliable, scalable, and automated spoilage detection (Ali et al., 2020; Ercolini et al., 2011; Hernández-Cázares et al., 2010; Hilgarth et al., 2018). Recent innovations are significantly enhancing the accuracy, robustness, and efficiency of microbial spoilage detection, making these systems increasingly viable for industrial applications in meat quality and safety monitoring. For instance, Recent studies have demonstrated the use of mass spectrometry to identify taxon-specific metabolic biomarkers across hundreds of bacterial species in >90% of samples, offering potential for direct detection of spoilage organisms in complex matrices such as meat (W. Chen et al., 2025). Similarly, experimental electrochemical methods, such as 3-(4,5-di-methylthiazol-2-yl)-2,5-

diphenyltetrazolium bromide (MTT)-based assays, have also shown potential for rapid bacterial viability estimation (within 10 minutes) in food safety applications, although they remain underutilized in meat spoilage detection systems (Ikeda et al., 2023). **Figure. 3** summarizes the framework for combining multi-omics platforms for training and developing the AI-based models for rapid and efficient meat spoilage detection.

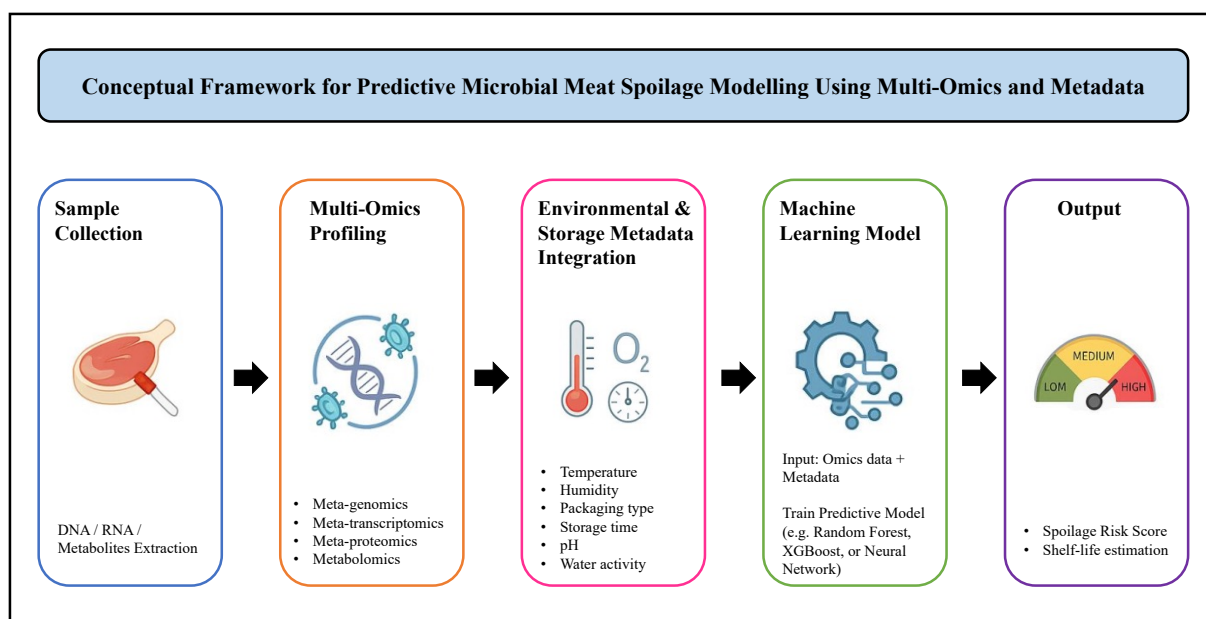


Figure. 3: Conceptual framework for the development and training the multi-omics integrated AI-based meat spoilage detection methods.

5. RECOMENDATIONS TO ADDRESS LIMITATIONS IN MICROBIAL DETECTION FOR MEAT SPOILAGE

5.1. Improved DNA extraction techniques:

DNA extraction is crucial for accurate microbial community analysis, with methods directly affecting the bacterial diversity observed. Flint et al. (2022) highlighted that the choice of extraction kit impacts the bacterial species identified in retail chicken breast (Flint et al., 2022). Cao & Song (2023) developed an enzymatic digestion-free method using magnetic nanoparticles, achieving 90% DNA recovery from high-fat meats, reducing bias (Cao and Song, 2023).

5.2. Faster and Long-read Sequencing Technologies:

Technologies like Oxford Nanopore's MinION and PacBio HiFi are advancing real-time DNA sequencing, essential for spoilage monitoring. These systems offer long-read sequencing to detect complex microbial communities, with MinION providing portability and PacBio resolving phage integration sites critical for spoilage prediction. While error rates and the costs remain challenging (Logsdon et al., 2021; Satam et al., 2023). But the accuracy of these platforms is improving, making them suitable for real-time spoilage detection (Zhang et al., 2020).

5.3. Development of spoilage-specific databases:

The advancement of metagenomic analyses in food science heavily relies on customized databases which enable spoilage-associated gene identification. Databases tailored to food microbiomes, such as the EU-funded CuratedFoodMetagenomicData (cFMD), are instrumental in identifying spoilage-associated genes. The cFMD database, for instance, consolidates over 50,000 metagenomes from diverse foods, enabling precise spoilage gene annotation with 95% specificity (Carlino et al., 2024). These resources provide invaluable tools for enhancing the accuracy of microbial spoilage detection.

5.4. Collaboration with Multi-Omics Approaches:

Integrating multi-omics approaches, including metagenomics, metabolomics, and proteomics, can offer a comprehensive view of spoilage. For instance, De Filippis et al. (2016) and Ferrocino et al. (2018) demonstrated how metabolomics coupled with metagenomics can link microbial clusters to changes in colour, pH, and flavour in fermented meats. Similarly, in another report, a holistic approach combining omics data (microbial, metabolomic, and sensory data) has provided new insights into the microbial ecology of spoilage in meat products (Ferrocino et al., 2023; Filippis et al., 2016). By analysing over 600 samples, researchers identified key spoilage microbes and their metabolic pathways, for *Leuconostoc* and *Lactococcus* species. This integrated method is highly accurate and provides a deeper understanding of the impact of production parameters (like packaging) on spoilage, offering valuable information for predicting spoilage scenarios in real-world meat production settings (Poirier et al., 2023). Despite the potential, challenges in data integration and high costs persist, requiring future research to focus on standardizing methodologies and developing cost-effective solutions (Valdés et al., 2022).

5.5. Cost reduction strategies, Scalability, and real-time monitoring

To reduce costs for small-scale meat processors, cloud-based bioinformatics platforms, like those described by D’Agostino et al. (2019), offer affordable metagenomic analysis (D’Agostino et al., 2019). While metagenomics provides in-depth microbial insights, VOC detection offers a faster, more scalable solution for real-time spoilage monitoring. Combining both approaches can ensure timely responses in the industry while maintaining comprehensive microbial characterization (Billington et al., 2022; Poirier et al., 2023).

Table 4: Key Fast Detection Methods, its Principles, and Application for Meat Spoilage

Method	Principle	Application	Key Studies / Citations
VOCs-Based Detection	Detection of volatile compounds (e.g., isopropanol, methanethiol, trimethylamine) produced during microbial spoilage.	Real-time monitoring using VOCs detections.	(Ercolini et al., 2011; Kergourlay et al., 2015)
Electronic Nose (E-nose)	Mimics mammalian olfaction using sensor arrays and pattern recognition algorithms.	VOC-based odour profiling of meat spoilage; machine learning integration for spoilage state classification.	(Modesti et al., 2021)
IoT-enabled electronic nose system	Detection of VOCs. Detect carbon dioxide, ammonia, and ethylene gases. Can also correlate VOCs with bacterial growth, particularly <i>Pseudomonas</i> spp. and aerobic bacteria.	Fast, accessible, and non-destructive method for spoilage detection, making it a promising tool for the food industry to ensure beef freshness.	(Damdam et al., 2023)

Non-enzymatic electrochemical sensor	Based on ZnO nanoparticles and porous graphene	Detect hypoxanthine (HXA), a key indicator of meat spoilage, Rapid, cost-effective, scalable and offers high sensitivity (0.14 μ M limit of detection)	(Le et al., 2024)
AI-Based Gas Sensor	Gas sensors combined with deep learning models such as convolutional neural networks (CNN)	Efficient, scalable, and fast. Can detect spoilage-related gases and image-based classification to determine meat freshness. Can achieve high accuracy of 99% for meat freshness.	(Bhuiyan et al., 2024)
AI and IoT-based sensor system	Combines gas sensors, cameras, LED indicators and deep learning (CNN)	Reliable, user-friendly, and fast. Can classify meat as fresh, semi-fresh, or rotten with 99.44% accuracy in freshness detection.	(Kim et al., 2024)

Laser Speckle Decorrelation	Measures dynamic speckle intensity patterns to detect the presence of live microorganisms optically and non-invasively.	Rapid pre-screening and bacterial activity detection in meat products.	(Hilgarth et al., 2018)
Smart Biosensors	Use of biological components (e.g., enzymes, antibodies, nucleic acids) and transducers to detect spoilage indicators or pathogens.	Specific detection of spoilage indicators (e.g., hypoxanthine) and pathogens (e.g., E. coli, Salmonella).	(Ali et al., 2020; Hernández-Cázares et al., 2010)

7. Comparison of Metagenomics with VOC Sensors and AI-based Detection Systems

Emerging technologies in meat spoilage detection, including electrochemical sensors, VOC sensors, and AI-driven systems, are driving a shift toward more accurate, robust, accessible, and most importantly scalable solutions. These innovations are improving the speed and reliability of spoilage detection, making it easier to monitor meat quality throughout the supply chain. The integration of IoT, deep learning, and omics data allows for earlier detection of spoilage with greater precision, while reducing detection time. Ultimately enhancing food safety, reducing waste, and improving overall meat quality management.

However, while AI-based systems and VOC sensors provide rapid, and real-time monitoring, they lack the taxonomical and functional resolution of unknown players. Therefore, other more time-consuming methods such as sequencing-based, meta-transcriptomic, and meta-proteomic techniques still play an essential role. These methods offer deep insights into microbial communities and spoilage mechanisms, making them invaluable for building the foundational knowledge required to improve and guide these new emerging technologies. Sequencing methods, for example, provide detailed information about microbial species and their metabolic capabilities. This knowledge is crucial for fine-tuning VOC and AI-based systems to detect and predict spoilage events more accurately. **Figure. 5** illustrates a comprehensive summary of slow and fast detection methods for meat spoilage.

Thus, while newer methods are highly effective and scalable, the evolution of other techniques remain necessary. They continue to provide essential data that enhance the performance of these emerging technologies, ensuring a more holistic and reliable approach to meat spoilage detection.

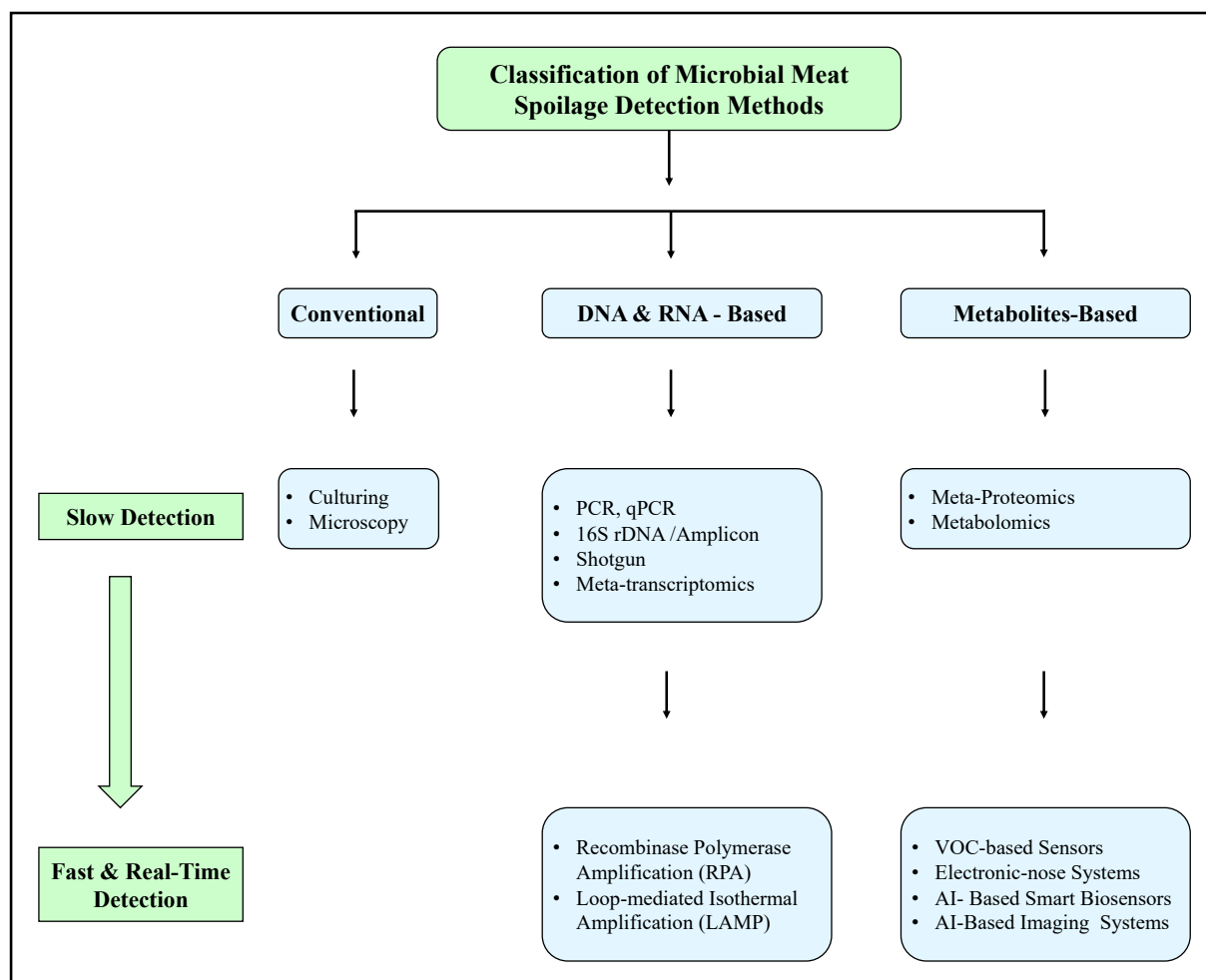


Figure. 5: The figure summarizes the microbial meat spoilage detection methods based on target inputs (e.g., DNA/RNA/metabolites) and time-taking considerations (e.g., slow, or fast).

CONCLUSION:

Meat spoilage is a multi-billion-dollar global crisis. Tackling it requires innovative solutions that meet the demands of a growing population and an increasingly fragile food system. This review has examined two decades of research and highlighted a key insight: current detection methods are reactive, fragmented, and not equipped to address the complex dynamics of modern meat spoilage.

Traditional culture-based techniques, while foundational, but cannot reveal the hidden microbial diversity driving spoilage. In contrast, metagenomics especially shotgun sequencing offers high-resolution insight into microbial communities and their spoilage-related functions. However, challenges remain in bringing these tools from research labs to industrial settings. Recent innovations, such as rapid DNA extraction methods, portable sequencers, and AI-integrated platforms, are narrowing this gap by reducing cost and detection time from days to just hours.

The future of spoilage control lies in hybrid systems that combine the depth of metagenomics with the speed of AI and IoT-based biosensors. For instance, integrating VOC e-noses with blockchain-based traceability platforms can predict spoilage up to five days in advance while ensuring supply chain transparency. Global efforts, including the EU's MASTER project and FAO-supported sequencing initiatives, are making these technologies more accessible especially in developing countries where nearly 30% of meat is lost after harvest.

To make this vision a reality, three urgent actions are needed:

Standardization: Develop unified protocols for DNA extraction, sequencing, and bioinformatics to ensure reproducibility across studies.

Database Curation: Build and maintain spoilage-specific genomic repositories, including climate-resilient strains and markers for spoilage and antimicrobial resistance.

Policy Support: Promote the adoption of AI-driven, multi-omics platforms through tax incentives, funding, and alignment with global food waste goals (e.g., UN SDG 12.3).

Improved detection methods help ensure meat reaches consumers safely. But they cannot prevent spoilage on their own. While oxidative spoilage can be managed by limiting air and light, and enzymatic degradation by chilling, microbial spoilage is far more complex. Its control requires robust detection. Sequencing methods are essential for mapping microbial growth and metabolic activity. These methods lay the groundwork for fast, real-time tools like VOC sensors, IoT systems, and AI-based detectors. While sequencing advances academic research, rapid detection systems empower industry. Together, they must work in harmony. This review is more than a summary, it is a call to action. By shifting from reactive testing to proactive, precision spoilage management, we can reduce up to \$1.3 trillion in annual losses, strengthen food security, and build resilient supply chains. The technologies exist. Now, science, industry, and policy must collaborate to bring this transformation to life.

References:

1. Abril, A.G., Calo-Mata, P., Villa, T.G., Böhme, K., Barros-Velázquez, J., Sánchez-Pérez, Á., Pazos, M., Carrera, M., 2024. Comprehensive shotgun proteomic characterization and virulence factors of seafood spoilage bacteria. *Food Chemistry* 448, 139045. <https://doi.org/10.1016/j.foodchem.2024.139045>
2. Addis, M., 2015. Major Causes Of Meat Spoilage and Preservation Techniques: A Review. *Food Science and Quality Management* 41, 101–114.
3. Ahmad Mir, S., Ahmad Masoodi, F., Raja, J., 2017. Influence of natural antioxidants on microbial load, lipid oxidation and sensorial quality of rista—A traditional meat product of India. *Food Bioscience* 20, 79–87. <https://doi.org/10.1016/j.fbio.2017.08.004>
4. Ali, L., Mohammed, M.U., Khan, M., Yousuf, A.H.B., Chowdhury, M.H., 2020. High-Quality Optical Ring Resonator-Based Biosensor for Cancer Detection. *IEEE Sensors Journal* 20, 1867–1875. <https://doi.org/10.1109/JSEN.2019.2950664>
5. Amaral, A.B., Silva, M.V. da, Lannes, S.C. da S., 2018. Lipid oxidation in meat: mechanisms and protective factors – a review. *Food Sci. Technol* 38, 1–15. <https://doi.org/10.1590/fst.32518>
6. André, S., Vallaes, T., Planchon, S., 2017. Spore-forming bacteria responsible for food spoilage. *Research in Microbiology, Beneficial and detrimental spore-formers* 168, 379–387. <https://doi.org/10.1016/j.resmic.2016.10.003>
7. Bahlinger, E., Dorn-In, S., Beindorf, P.-M., Mang, S., Kaltner, F., Gottschalk, C., Gareis, M., Schwaiger, K., 2021. Development of two specific multiplex qPCRs to determine amounts of *Pseudomonas*, *Enterobacteriaceae*, *Brochothrix thermosphacta* and *Staphylococcus* in meat and heat-treated meat products. *International Journal of Food Microbiology* 337, 108932. <https://doi.org/10.1016/j.ijfoodmicro.2020.108932>
8. Barcenilla, C., Cobo-Díaz, J.F., Puente, A., Valentino, V., De Filippis, F., Ercolini, D., Carlino, N., Pinto, F., Segata, N., Prieto, M., López, M., Alvarez-Ordóñez, A., 2024. In-depth characterization of food and environmental microbiomes across different meat processing plants. *Microbiome* 12, 199. <https://doi.org/10.1186/s40168-024-01856-3>

9. Bayrakal, G.M., Çiftçioğlu, G., 2023. Spoilage detection of smart packaged chicken meat by ddPCR. *Eur Food Res Technol* 249, 2635–2645. <https://doi.org/10.1007/s00217-023-04321-x>
10. Bhuiyan, Z.W., Haider, S.A.R., Haque, A., Uddin, M.R., Hasan, M., 2024. IoT Based Meat Freshness Classification Using Deep Learning. *IEEE Access* 12, 196047–196069. <https://doi.org/10.1109/ACCESS.2024.3520029>
11. Binson, V.A., Thomas, S., 2023. The Development of a Mobile E-Nose System for Real-Time Beef Quality Monitoring and Spoilage Detection. *Engineering Proceedings* 56, 256. <https://doi.org/10.3390/ASEC2023-15960>
12. Borch, E., Kant-Muermans, M.-L., Blixt, Y., 1996. Bacterial spoilage of meat and cured meat products. *International Journal of Food Microbiology, Specific Spoilage Organisms* 33, 103–120. [https://doi.org/10.1016/0168-1605\(96\)01135-X](https://doi.org/10.1016/0168-1605(96)01135-X)
13. Broekaert, K., Heyndrickx, M., Herman, L., Devlieghere, F., Vlaemynck, G., 2013. Molecular identification of the microbiota of peeled and unpeeled brown shrimp (*Crangon crangon*) during storage on ice and at 7.5 °C. *Food Microbiology* 36, 123–134. <https://doi.org/10.1016/j.fm.2013.04.009>
14. Cao, Y., Song, X., 2023. Meat Authenticity Made Easy: DNA Extraction-Free Rapid Onsite Detection of Duck and Pork Ingredients in Beef and Lamb Using Dual-Recombinase-Aided Amplification and Multiplex Lateral Flow Strips. *J. Agric. Food Chem.* 71, 14782–14794. <https://doi.org/10.1021/acs.jafc.3c03259>
15. Carlino, N., Blanco-Míguez, A., Punčochář, M., Mengoni, C., Pinto, F., Tatti, A., Manghi, P., Armanini, F., Avagliano, M., Barcenilla, C., Breselge, S., Cabrera-Rubio, R., Calvete-Torre, I., Coakley, Mairéad, Cobo-Díaz, José F., Filippis, F.D., Dey, H., Leech, J., Klaassens, E.S., Knobloch, S., O’Neil, D., Quijada, N.M., Sabater, C., Skirnisdóttir, S., Valentino, V., Walsh, L., Alvarez-Ordóñez, A., Alvarez, P., Antonielli, L., Arendt, E., Armanini, F., Aubry, A., Baelum, J., Barcenilla, C., Belanche, A., Benavent-Gil, Y., Blake, T., Blanco-Míguez, A., Bongoni, R., Boyer, M., Brennan, F., Breselge, S., Briem, H., Butler, D., Calvete-Torre, I., Carballo, O.C., Cardinal, M., Carlino, N., Chervaux, C., Chopin, C., Clotaire, N., Coakley, Mairead, Cobo-Díaz, José Francisco, Codd, J., Conroy, S., Corral-Jara, K.F., Corral-Jara, K.-F., Cotter, P.D., Coyne, G., Creevey, C., Cuevas, P.D., Curran, B., Delgado, S., Derde, L., Derrien, M.,

- Ercolini, D., Exposito, R.G., Fernández, M.M.L., Filippis, F.D., Fordham, D., Galy, H., Gavriilidou, A., Gunnarsson, O., Hanson, B., Hermes, G., Huang, R., Huws, S., Ikoyi, I., Jaeger, A., Jeffery, I., Jérôme, M., Juan, P.-A., Kenny, D., Kers, A., Khinouché, K.-F., Kirwan, S., Klaassens, E.S., Knobloch, S., Kolbeinsson, K., Kolypczuk, L., Kostic, T., Ledda, F., Leech, J., Lehmann, D., Leroi, F., Lewis, E., Ley, J., Lucic, E., Lynch, K., Mace, S., MacLaren-Lee, I., Sanchez, L.M. de, Marchart, J., Margolles, A., Marteinsson, V.T., Masetti, G., McGovern, F., McHugh, N., McLoughlin, S., Meehan, D., Mølbak, L., Monin, T., Moreno, J., Morgavi, D., Morrison, S., Muench, S., Neves, A.R.R., Neylon, E., Nyhan, L., O’Kelly, R., O’Neil, D., O’Toole, P., Ortiz-Chura, A., Palma, J.M., Pasolli, E., Passerini, D., Pastar, M., Pinto, F., Pirovano, W., Plans, O., Policht, M., Pop, A., Pop, B., Popova, M., Prieto, M., Quijada, N.M., Reiss, A., Romero, P., Ruas-Madiedo, P., Rubino, F., Rubio, R.C., Ruiz, L., Ryan, A., Ryan, C., Sabater, C., Sahin, A., Salaun, C., Santos, F.G., Schneider, C., Segata, N., Selberherr, E., Sessitsch, A., Skirnisdóttir, S., Smidt, H., Smith, P., Sprenger-Haussels, M., Tapio, I., Tap, J., Valentino, V., Wagner, M., Walsh, A., Walsh, L., Waters, S.M., Willcocks, S., Yáñez-Ruiz, D.R., Yan, T., Yap, M., Zannini, E., Zuliani, V., Alvarez-Ordóñez, A., Asnicar, F., Fackelmann, G., Heidrich, V., Margolles, A., Marteinsson, V.T., Stabelli, O.R., Wagner, M., Ercolini, D., Cotter, P.D., Segata, N., Pasolli, E., 2024. Unexplored microbial diversity from 2,500 food metagenomes and links with the human microbiome. *Cell* 187, 5775-5795.e15. <https://doi.org/10.1016/j.cell.2024.07.039>
16. Carrizosa, E., Benito, M.J., Ruiz-Moyano, S., Hernández, A., Villalobos, M. del C., Martín, A., Córdoba, M. de G., 2017. Bacterial communities of fresh goat meat packaged in modified atmosphere. *Food Microbiology* 65, 57–63. <https://doi.org/10.1016/j.fm.2017.01.023>
17. Casaburi, A., De Filippis, F., Villani, F., Ercolini, D., 2014. Activities of strains of *Brochothrix thermosphacta* *in vitro* and in meat. *Food Research International* 62, 366–374. <https://doi.org/10.1016/j.foodres.2014.03.019>
18. Casaburi, A., Piombino, P., Nychas, G.-J., Villani, F., Ercolini, D., 2015. Bacterial populations and the volatilome associated to meat spoilage. *Food Microbiology, Spoilers, wonder spores and diehard microorganisms: New insights to integrate these super foes in food spoilage risk management* 45, 83–102. <https://doi.org/10.1016/j.fm.2014.02.002>

19. Cervený, J., Meyer, J.D., Hall, P.A., 2009. Microbiological Spoilage of Meat and Poultry Products, in: Sperber, W.H., Doyle, M.P. (Eds.), *Compendium of the Microbiological Spoilage of Foods and Beverages*. Springer, New York, NY, pp. 69–86. https://doi.org/10.1007/978-1-4419-0826-1_3
20. Chen, N., Fu, W., Jiang, L., Zhang, N., Mu, Y., Su, W., 2025. Metatranscriptomics reveals the effect of fermentation by *Pediococcus acidilactici* and *Rhizopus oryzae* on the quality of mutton sausages and the mechanism of odor metabolism. *Food Chemistry* 477, 143472. <https://doi.org/10.1016/j.foodchem.2025.143472>
21. Chen, W., Qiu, M., Paizs, P., Sadowski, M., Ramonaite, T., Zborovsky, L., Mejias-Luque, R., Janßen, K.-P., Kinross, J., Goldin, R.D., Rebec, M., Liebeke, M., Takats, Z., McKenzie, J.S., Strittmatter, N., 2025. Universal, untargeted detection of bacteria in tissues using metabolomics workflows. *Nat Commun* 16, 165. <https://doi.org/10.1038/s41467-024-55457-7>
22. Chen, Y., Bassey, A.P., Bai, Y., Teng, S., Zhou, G., Ye, K., 2022. Synergistic Effect of Static Magnetic Field and Modified Atmosphere Packaging in Controlling Blown Pack Spoilage in Meatballs. *Foods* 11, 1374. <https://doi.org/10.3390/foods11101374>
23. Cobo-Díaz, J.F., Alvarez-Molina, A., Alexa, E.A., Walsh, C.J., Mencía-Ares, O., Puente-Gómez, P., Likotrafiti, E., Fernández-Gómez, P., Prieto, B., Crispie, F., Ruiz, L., González-Raurich, M., López, M., Prieto, M., Cotter, P., Alvarez-Ordóñez, A., 2021. Microbial colonization and resistome dynamics in food processing environments of a newly opened pork cutting industry during 1.5 years of activity. *Microbiome* 9, 204. <https://doi.org/10.1186/s40168-021-01131-9>
24. da Silva, L.O., de Carvalho, G.G.P., Tosto, M.S.L., Lima, V.G.O., Cirne, L.G.A., de Araújo, M.L.G.M.L., Pina, D. dos S., Leite, V.M., Rodrigues, C.S., Mesquita, B.M.A. de C., 2021. Effects of palm kernel cake in high-concentrate diets on carcass traits and meat quality of feedlot goats. *Livestock Science* 246, 104456. <https://doi.org/10.1016/j.livsci.2021.104456>
25. D’Agostino, D., Morganti, L., Corni, E., Cesini, D., Merelli, I., 2019. Combining Edge and Cloud computing for low-power, cost-effective metagenomics analysis. *Future Generation Computer Systems* 90, 79–85. <https://doi.org/10.1016/j.future.2018.07.036>

-
26. Damdam, A.N., Ozay, L.O., Ozcan, C.K., Alzahrani, A., Helabi, R., Salama, K.N., 2023. IoT-Enabled Electronic Nose System for Beef Quality Monitoring and Spoilage Detection. *Foods* 12, 2227. <https://doi.org/10.3390/foods12112227>
27. Dave, D., Ghaly, A.E., 2011. Meat Spoilage Mechanisms and Preservation Techniques: A Critical Review. *American Journal of Agricultural and Biological Sciences* 6, 486–510. <https://doi.org/10.3844/ajabssp.2011.486.510>
28. De Filippis, F., La Stora, A., Villani, F., Ercolini, D., 2018. Strain-Level Diversity Analysis of *Pseudomonas fragi* after In Situ Pangenome Reconstruction Shows Distinctive Spoilage-Associated Metabolic Traits Clearly Selected by Different Storage Conditions. *Applied and Environmental Microbiology* 85, e02212-18. <https://doi.org/10.1128/AEM.02212-18>
29. De Filippis, F., Valentino, V., Alvarez-Ordóñez, A., Cotter, P.D., Ercolini, D., 2021. Environmental microbiome mapping as a strategy to improve quality and safety in the food industry. *Current Opinion in Food Science* 38, 168–176. <https://doi.org/10.1016/j.cofs.2020.11.012>
30. De Filippis, F., Valentino, V., Yap, M., Cabrera-Rubio, R., Barcenilla, C., Carlino, N., Cobo-Díaz, J.F., Quijada, N.M., Calvete-Torre, I., Ruas-Madiedo, P., Sabater, C., Sequino, G., Pasolli, E., Wagner, M., Margolles, A., Segata, N., Álvarez-Ordóñez, A., Cotter, P.D., Ercolini, D., 2024. Microbiome mapping in dairy industry reveals new species and genes for probiotic and bioprotective activities. *npj Biofilms Microbiomes* 10, 1–16. <https://doi.org/10.1038/s41522-024-00541-5>
31. Ercolini, D., Ferrocino, I., Nasi, A., Ndagijimana, M., Vernocchi, P., La Stora, A., Laghi, L., Mauriello, G., Guerzoni, M.E., Villani, F., 2011. Monitoring of Microbial Metabolites and Bacterial Diversity in Beef Stored under Different Packaging Conditions. *Applied and Environmental Microbiology* 77, 7372–7381. <https://doi.org/10.1128/AEM.05521-11>
32. EU Commission Regulation (EC) No 853/2004, E., n.d. Regulation (EC) No 853/2004 of the European Parliament and of the Council of 29 April 2004 laying down specific hygiene rules for food of animal origin [WWW Document]. <https://webarchive.nationalarchives.gov.uk/eu-exit/https://eur-lex.europa.eu/legal->

- content/EN/TXT/?uri=CELEX:02004R0853-20190726. URL <https://www.legislation.gov.uk/eur/2004/853/annex/III> (accessed 5.10.25).
33. FAO, 2022. World Agriculture: Towards 2015/2030 - An FAO perspective [WWW Document]. URL <https://www.fao.org/4/y4252e/y4252e05b.htm?> (accessed 5.10.25).
34. Ferrocino, I., Rantsiou, K., McClure, R., Kostic, T., de Souza, R.S.C., Lange, L., FitzGerald, J., Kriaa, A., Cotter, P., Maguin, E., Schelkle, B., Schlöter, M., Berg, G., Sessitsch, A., Cocolin, L., Consortium, T.M., 2023. The need for an integrated multi-OMICs approach in microbiome science in the food system. *Comprehensive Reviews in Food Science and Food Safety* 22, 1082–1103. <https://doi.org/10.1111/1541-4337.13103>
35. Filippis, F.D., Pellegrini, N., Vannini, L., Jeffery, I.B., Stora, A.L., Laghi, L., Serrazanetti, D.I., Cagno, R.D., Ferrocino, I., Lazzi, C., Turrioni, S., Cocolin, L., Brigidi, P., Neviani, E., Gobetti, M., O'Toole, P.W., Ercolini, D., 2016. High-level adherence to a Mediterranean diet beneficially impacts the gut microbiota and associated metabolome. *Gut* 65, 1812–1821. <https://doi.org/10.1136/gutjnl-2015-309957>
36. Fletcher, B., Mullane, Keegan, Platts, Phoebe, Todd, Ethan, Power, Aoife, Roberts, Jessica, Chapman, James, Cozzolino, Daniel, and Chandra, S., 2018. Advances in meat spoilage detection: A short focus on rapid methods and technologies. *CyTA - Journal of Food* 16, 1037–1044. <https://doi.org/10.1080/19476337.2018.1525432>
37. Flint, A., Laidlaw, A., Li, L., Raitt, C., Rao, M., Cooper, A., Weedmark, K., Carrillo, C., Tamber, S., 2022. Choice of DNA extraction method affects detection of bacterial taxa from retail chicken breast. *BMC Microbiol* 22, 230. <https://doi.org/10.1186/s12866-022-02650-7>
38. Fu, L., Wang, C., Liu, N., Ma, A., Wang, Y., 2018. *Quorum sensing* system-regulated genes affect the spoilage potential of *Shewanella baltica*. *Food Research International* 107, 1–9. <https://doi.org/10.1016/j.foodres.2018.01.067>
39. Geiker, N.R.W., Bertram, H.C., Mejbørn, H., Dragsted, L.O., Kristensen, L., Carrascal, J.R., Bügel, S., Astrup, A., 2021. Meat and Human Health—Current Knowledge and Research Gaps. *Foods* 10, 1556. <https://doi.org/10.3390/foods10071556>

-
40. Gladding, T.L., Gwyther, C.L., 2017. A study of the potential release of bioaerosols from containers as a result of reduced frequency residual waste collections. *Science of The Total Environment* 576, 481–489. <https://doi.org/10.1016/j.scitotenv.2016.10.060>
41. González-Fandos, E., Martínez Laorden, A., Condón Usón, S., Serrano Andrés, M.J., 2025. Growth and Diversity of Spoiling and Foodborne Bacteria in Poultry Hamburgers in Modified Atmosphere and with Sulfites During Shelf Life. *Microorganisms* 13, 754. <https://doi.org/10.3390/microorganisms13040754>
42. Gram, L., Dalgaard, P., 2002. Fish spoilage bacteria – problems and solutions. *Current Opinion in Biotechnology* 13, 262–266. [https://doi.org/10.1016/S0958-1669\(02\)00309-9](https://doi.org/10.1016/S0958-1669(02)00309-9)
43. Grützke, J., Malorny, B., Hammerl, J.A., Busch, A., Tausch, S.H., Tomaso, H., Deneke, C., 2019. Fishing in the Soup – Pathogen Detection in Food Safety Using Metabarcoding and Metagenomic Sequencing. *Front. Microbiol.* 10. <https://doi.org/10.3389/fmicb.2019.01805>
44. Gu, M., Zhang, D., Li, C., Ren, Y., Song, G., Chen, L., Li, S., Zheng, X., 2024. In-depth metaproteomics analysis reveals the protein profile and metabolism characteristics in pork during refrigerated storage. *Food Chemistry* 459, 140149. <https://doi.org/10.1016/j.foodchem.2024.140149>
45. Harada, T., Dang, V.C., Nguyen, D.P., Nguyen, T.A.D., Sakamoto, M., Ohkuma, M., Motooka, D., Nakamura, S., Uchida, K., Jinnai, M., Yonogi, S., Kawahara, R., Kanki, M., Kawai, T., Kumeda, Y., Yamamoto, Y., 2016. *Enterococcus saigonensis* sp. nov., isolated from retail chicken meat and liver. *International Journal of Systematic and Evolutionary Microbiology* 66, 3779–3785. <https://doi.org/10.1099/ijsem.0.001264>
46. Hernández-Cázares, A.S., Aristoy, M.-C., Toldrá, F., 2010. Hypoxanthine-based enzymatic sensor for determination of pork meat freshness. *Food Chemistry* 123, 949–954. <https://doi.org/10.1016/j.foodchem.2010.04.066>
47. Hilgarth, M., Behr, J., Vogel, R.F., 2018. Monitoring of spoilage-associated microbiota on modified atmosphere packaged beef and differentiation of psychrophilic and psychrotrophic strains. *Journal of Applied Microbiology* 124, 740–753. <https://doi.org/10.1111/jam.13669>

-
48. Hugas, M., Garriga, M., Aymerich, M.T., 2003. Functionalty of enterococci in meat products. *International Journal of Food Microbiology, Enterococci in Foods. Functional and Safety Aspects* 88, 223–233. [https://doi.org/10.1016/S0168-1605\(03\)00184-3](https://doi.org/10.1016/S0168-1605(03)00184-3)
49. Hultman, J., Johansson, P., Björkroth, J., 2020. Longitudinal Metatranscriptomic Analysis of a Meat Spoilage Microbiome Detects Abundant Continued Fermentation and Environmental Stress Responses during Shelf Life and Beyond. *Applied and Environmental Microbiology* 86, e01575-20. <https://doi.org/10.1128/AEM.01575-20>
50. Hwang, B.K., Choi, H., Choi, S.H., Kim, B.-S., 2020. Analysis of Microbiota Structure and Potential Functions Influencing Spoilage of Fresh Beef Meat. *Front. Microbiol.* 11. <https://doi.org/10.3389/fmicb.2020.01657>
51. Ikeda, H., Tokonami, A., Nishii, S., Shan, X., Yamamoto, Y., Sadanaga, Y., Chen, Z., Shiigi, H., 2023. Evaluation of Bacterial Activity Based on the Electrochemical Properties of Tetrazolium Salts. *Anal. Chem.* 95, 12358–12364. <https://doi.org/10.1021/acs.analchem.3c01871>
52. Indio, V., Oliveri, C., Lucchi, A., Savini, F., Gonzales-Barron, U., Skandamis, P., Achemchem, F., Manfreda, G., Serraino, A., De Cesare, A., 2024. Shotgun metagenomic investigation of foodborne pathogens and antimicrobial resistance genes in artisanal fermented meat products from the Mediterranean area. *Ital J Food Saf* 13, 12210. <https://doi.org/10.4081/ijfs.2024.12210>
53. Jérôme, M., Passerini, D., Chevalier, F., Marchand, L., Leroi, F., Macé, S., 2022. Development of a rapid qPCR method to quantify lactic acid bacteria in cold-smoked salmon. *International Journal of Food Microbiology* 363, 109504. <https://doi.org/10.1016/j.ijfoodmicro.2021.109504>
54. Karwowska, M., Łaba, S., Szczepański, K., 2021. Food Loss and Waste in Meat Sector—Why the Consumption Stage Generates the Most Losses? *Sustainability* 13, 6227. <https://doi.org/10.3390/su13116227>
55. Katare, B., Wang, H.H., Lawing, J., Hao, N., Park, T., Wetzstein, M., 2020. Toward Optimal Meat Consumption. *American Journal of Agricultural Economics* 102, 662–680. <https://doi.org/10.1002/ajae.12016>

56. Katiyo, W., de Kock, H.L., Coorey, R., Buys, E.M., 2020. Sensory implications of chicken meat spoilage in relation to microbial and physicochemical characteristics during refrigerated storage. *LWT* 128, 109468. <https://doi.org/10.1016/j.lwt.2020.109468>
57. Kergourlay, G., Taminiau, B., Daube, G., Champomier Vergès, M.-C., 2015. Metagenomic insights into the dynamics of microbial communities in food. *International Journal of Food Microbiology*, Special Issue: FoodMicro 2014, 24th International ICFMH Conference, From single cells to functions of consortia in food microbiology, 1 - 4 September, 2014, Nantes, France. 213, 31–39. <https://doi.org/10.1016/j.ijfoodmicro.2015.09.010>
58. Kim, D.-E., Mai, N.-D., Chung, W.-Y., 2024. AIoT-Based Meat Quality Monitoring Using Camera and Gas Sensor With Wireless Charging. *IEEE Sensors Journal* 24, 7317–7324. <https://doi.org/10.1109/JSEN.2023.3328915>
59. Kim, S., Lee, J.Y., Jeong, Y., Kang, C.-H., 2022. Antioxidant Activity and Probiotic Properties of Lactic Acid Bacteria. *Fermentation* 8, 29. <https://doi.org/10.3390/fermentation8010029>
60. Kolbeck, Sandra, Abele, M., Hilgarth, M., Vogel, R.F., 2021a. Comparative Proteomics Reveals the Anaerobic Lifestyle of Meat-Spoiling *Pseudomonas* Species. *Front. Microbiol.* 12. <https://doi.org/10.3389/fmicb.2021.664061>
61. Kolbeck, S., Hilgarth, M., Vogel, R.F., 2021. Proof of concept: predicting the onset of meat spoilage by an integrated oxygen sensor spot in MAP packages. *Letters in Applied Microbiology* 73, 39–45. <https://doi.org/10.1111/lam.13473>
62. Kolbeck, Sandra, Kienberger, H., Kleigrew, K., Hilgarth, M., Vogel, R.F., 2021b. Effect of high levels of CO₂ and O₂ on membrane fatty acid profile and membrane physiology of meat spoilage bacteria. *Eur Food Res Technol* 247, 999–1011. <https://doi.org/10.1007/s00217-020-03681-y>
63. Lawaniya, S.D., Awasthi, A., Menezes, P.W., Awasthi, K., 2025. Detection of Foodborne Pathogens Through Volatile Organic Compounds Sensing via Metal Oxide Gas Sensors. *Advanced Sensor Research* 4, 2400101. <https://doi.org/10.1002/adsr.202400101>

64. Le, N.T.H., Viet, N.X., Anh, N.V., Bach, T.N., Thu, P.T., Ngoc, N.T., Manh, D.H., Ky, V.H., Lam, V.D., Kodelov, V., Von Gratowski, S., Binh, N.H., Anh, T.X., 2024. Non-enzymatic electrochemical sensor based on ZnO nanoparticles/porous graphene for the detection of hypoxanthine in pork meat. *AIP Advances* 14, 025034. <https://doi.org/10.1063/5.0190293>
65. Lee, D.Y., Lee, S.Y., Jo, C., Yoon, Y., Jeong, J.Y., Hur, S.J., 2021. Effect on health from consumption of meat and meat products. *J Anim Sci Technol* 63, 955–976. <https://doi.org/10.5187/jast.2021.e101>
66. Li, X., Babol, J., Bredie, W.L.P., Nielsen, B., Tománková, J., Lundström, K., 2014. A comparative study of beef quality after ageing *longissimus* muscle using a dry ageing bag, traditional dry ageing or vacuum package ageing. *Meat Science* 97, 433–442. <https://doi.org/10.1016/j.meatsci.2014.03.014>
67. Liao, R., Xia, Q., Zhou, C., Geng, F., Wang, Y., Sun, Y., He, J., Pan, D., Cao, J., 2022. LC-MS/MS-based metabolomics and sensory evaluation characterize metabolites and texture of normal and spoiled dry-cured hams. *Food Chemistry* 371, 131156. <https://doi.org/10.1016/j.foodchem.2021.131156>
68. Logsdon, G.A., Vollger, M.R., Hsieh, P., Mao, Y., Liskovych, M.A., Koren, S., Nurk, S., Mercuri, L., Dishuck, P.C., Rhie, A., de Lima, L.G., Dvorkina, T., Porubsky, D., Harvey, W.T., Mikheenko, A., Bzikadze, A.V., Kremitzki, M., Graves-Lindsay, T.A., Jain, C., Hoekzema, K., Murali, S.C., Munson, K.M., Baker, C., Sorensen, M., Lewis, A.M., Surti, U., Gerton, J.L., Larionov, V., Ventura, M., Miga, K.H., Phillippy, A.M., Eichler, E.E., 2021. The structure, function and evolution of a complete human chromosome 8. *Nature* 593, 101–107. <https://doi.org/10.1038/s41586-021-03420-7>
69. Lupton, S.J., O’Keefe, Margaret, Muñoz-Ortiz, Jorge G., Clinch, Nelson, and Basu, P., 2017. Survey of polychlorinated dibenzo-p-dioxins, polychlorinated dibenzofurans and non-ortho-polychlorinated biphenyls in US meat and poultry, 2012–13: toxic equivalency levels, patterns, temporal trends and implications. *Food Additives & Contaminants: Part A* 34, 1970–1981. <https://doi.org/10.1080/19440049.2017.1340674>
70. Macé, S., Cornet, J., Chevalier, F., Cardinal, M., Pilet, M.-F., Dousset, X., Joffraud, J.-J., 2012. Characterisation of the spoilage microbiota in raw salmon (*Salmo salar*) steaks stored under vacuum or modified atmosphere packaging combining conventional

- methods and PCR–TTGE. *Food Microbiology* 30, 164–172. <https://doi.org/10.1016/j.fm.2011.10.013>
71. Mahmud, J., Muranyi, P., Salmieri, S., Lacroix, M., 2023. Optimization of a natural antimicrobial formulation against potential meat spoilage bacteria and food-borne pathogens: Mixture design methodology and predictive modeling. *Microbial Pathogenesis* 176, 106000. <https://doi.org/10.1016/j.micpath.2023.106000>
72. Marmion, M., Soro, A.B., Whyte, P., Scannell, A.G.M., 2023. A culture-based assessment of the microbiota of conventional and free-range chicken meat from Irish processing facilities. *Food Microbiology* 114, 104306. <https://doi.org/10.1016/j.fm.2023.104306>
73. Modesti, M., Taglieri, I., Bianchi, A., Tonacci, A., Sansone, F., Bellincontro, A., Venturi, F., Sanmartin, C., 2021. E-Nose and Olfactory Assessment: Teamwork or a Challenge to the Last Data? The Case of Virgin Olive Oil Stability and Shelf Life. *Applied Sciences* 11, 8453. <https://doi.org/10.3390/app11188453>
74. Møretørø, T., Moen, B., Heir, E., Hansen, A.Å., Langsrud, S., 2016. Contamination of salmon fillets and processing plants with spoilage bacteria. *International Journal of Food Microbiology* 237, 98–108. <https://doi.org/10.1016/j.ijfoodmicro.2016.08.016>
75. Murhekar, S., Wright, M.H., Greene, A.C., Brownlie, J.C., Cock, I.E., 2017. Inhibition of *Shewanella* spp. growth by *Syzygium australe* and *Syzygium luehmannii* extracts: natural methods for the prevention of fish spoilage. *J Food Sci Technol* 54, 3314–3326. <https://doi.org/10.1007/s13197-017-2782-6>
76. Nychas, G.-J.E., Skandamis, P.N., Tassou, C.C., Koutsoumanis, K.P., 2008. Meat spoilage during distribution. *Meat Science, Symposium on Meat safety: From Abattoir to Consumer* 78, 77–89. <https://doi.org/10.1016/j.meatsci.2007.06.020>
77. Odeyemi, O.A., Alegbeleye, O.O., Strateva, M., Stratev, D., 2020. Understanding spoilage microbial community and spoilage mechanisms in foods of animal origin. *Comprehensive Reviews in Food Science and Food Safety* 19, 311–331. <https://doi.org/10.1111/1541-4337.12526>
78. Oduoye, M.O., Olaide, Z., Adegoke, A.A., Agbaje, M.T., Akilimali, A., Lawal, M., 2024. The Roles of Redox and Enzymatic Processes in Food Spoilage in the Global

- South, in: Ogwu, M.C., Izah, S.C., Ntuli, N.R. (Eds.), Food Safety and Quality in the Global South. Springer Nature, Singapore, pp. 601–624. https://doi.org/10.1007/978-981-97-2428-4_19
79. OECD, FAO, 2023. OECD-FAO Agricultural Outlook 2023-2032 [WWW Document]. OECD-FAO Agricultural Outlook 2023-2032. URL https://www.oecd.org/en/publications/oecd-fao-agricultural-outlook-2023-2032_08801ab7-en/full-report/meat_7b036d52.html (accessed 5.10.25).
80. Palevich, N., Palevich, F.P., Maclean, P.H., Altermann, E., Gardner, A., Burgess, S., Mills, J., Brightwell, G., 2021. Comparative genomics of *Clostridium* species associated with vacuum-packed meat spoilage. Food Microbiology 95, 103687. <https://doi.org/10.1016/j.fm.2020.103687>
81. Papuc, C., Goran, G.V., Predescu, C.N., Nicorescu, V., 2017. Mechanisms of Oxidative Processes in Meat and Toxicity Induced by Postprandial Degradation Products: A Review. Comprehensive Reviews in Food Science and Food Safety 16, 96–123. <https://doi.org/10.1111/1541-4337.12241>
82. Parmley, E.J., Pintar, K., Majowicz, S., Avery, B., Cook, A., Jokinen, C., Gannon, V., Lapen, D.R., Topp, E., Edge, T.A., Gilmour, M., Pollari, F., Reid-Smith, R., Irwin, R., 2013. A Canadian Application of One Health: Integration of Salmonella Data from Various Canadian Surveillance Programs (2005–2010). Foodborne Pathogens and Disease 10, 747–756. <https://doi.org/10.1089/fpd.2012.1438>
83. Patarata, L., Novais, M., Fraqueza, M.J., Silva, J.A., 2020. Influence of Meat Spoilage Microbiota Initial Load on the Growth and Survival of Three Pathogens on a Naturally Fermented Sausage. Foods 9, 676. <https://doi.org/10.3390/foods9050676>
84. Pellissery, A.J., Vinayamohan, P.G., Amalaradjou, M.A.R., Venkitanarayanan, K., 2020. Chapter 17 - Spoilage bacteria and meat quality, in: Biswas, A.K., Mandal, P.K. (Eds.), Meat Quality Analysis. Academic Press, pp. 307–334. <https://doi.org/10.1016/B978-0-12-819233-7.00017-3>
85. Poirier, S., Coeuret, G., Champomier-Vergès, M.-C., Desmonts, M.-H., Werner, D., Feurer, C., Frémaux, B., Guillou, S., Luong, N.-D.M., Rué, O., Loux, V., Zagorec, M., Chaillou, S., on behalf of the ANR Redlosses Consortium Group, 2023. Holistic

- integration of omics data reveals the drivers that shape the ecology of microbial meat spoilage scenarios. *Front. Microbiol.* 14. <https://doi.org/10.3389/fmicb.2023.1286661>
86. Poore, J., Nemecek, T., 2018. Reducing food's environmental impacts through producers and consumers. *Science* 360, 987–992. <https://doi.org/10.1126/science.aag0216>
87. Rahkila, R., Johansson, P., Säde, E., Björkroth, J., 2011. Identification of Enterococci from Broiler Products and a Broiler Processing Plant and Description of *Enterococcus viikkiensis* sp. nov. *Applied and Environmental Microbiology* 77, 1196–1203. <https://doi.org/10.1128/AEM.02412-10>
88. Ramanathan, R., Lambert, L.H., Nair, M.N., Morgan, B., Feuz, R., Mafi, G., Pfeiffer, M., 2022. Economic Loss, Amount of Beef Discarded, Natural Resources Wastage, and Environmental Impact Due to Beef Discoloration. *Meat and Muscle Biology* 6. <https://doi.org/10.22175/mmb.13218>
89. Rao, A.S., Deka, S.P., More, S.S., Nair, A., More, V.S., Ananthjaraju, K.S., 2021. A Comprehensive Review on Different Microbial-Derived Pigments and Their Multipurpose Activities, in: Vaishnav, A., Choudhary, D.K. (Eds.), *Microbial Polymers: Applications and Ecological Perspectives*. Springer, Singapore, pp. 479–519. https://doi.org/10.1007/978-981-16-0045-6_20
90. Raposo, A., Pérez, E., de Faria, C.T., Ferrús, M.A., Carrascosa, C., 2016. Food Spoilage by *Pseudomonas* spp.—An Overview, in: *Foodborne Pathogens and Antibiotic Resistance*. John Wiley & Sons, Ltd, pp. 41–71. <https://doi.org/10.1002/9781119139188.ch3>
91. Rawat, S., 2015. Food Spoilage: Microorganisms and their prevention. *Asian Journal of Plant Science and Research* 5, 47–56.
92. Rouger, A., Tresse, O., Zagorec, M., 2017. Bacterial Contaminants of Poultry Meat: Sources, Species, and Dynamics. *Microorganisms* 5, 50. <https://doi.org/10.3390/microorganisms5030050>
93. Sabater, C., Cobo-Díaz, J.F., Álvarez-Ordóñez, A., Ruas-Madiedo, P., Ruiz, L., Margolles, A., 2021. Novel methods of microbiome analysis in the food industry. *Int Microbiol* 24, 593–605. <https://doi.org/10.1007/s10123-021-00215-8>

-
94. Saenz-García, C.E., Castañeda-Serrano, P., Mercado Silva, E.M., Alvarado, C.Z., Nava, G.M., 2020. Insights into the Identification of the Specific Spoilage Organisms in Chicken Meat. *Foods* 9, 225. <https://doi.org/10.3390/foods9020225>
95. Satam, H., Joshi, K., Mangrolia, U., Waghoo, S., Zaidi, G., Rawool, S., Thakare, R.P., Banday, S., Mishra, A.K., Das, G., Malonia, S.K., 2023. Next-Generation Sequencing Technology: Current Trends and Advancements. *Biology* 12, 997. <https://doi.org/10.3390/biology12070997>
96. Sequino, G., Cobo-Díaz, J.F., Valentino, V., Tassou, C., Volpe, S., Torrieri, E., Nychas, G.-J., Álvarez Ordóñez, A., Ercolini, D., De Filippis, F., 2024. Microbiome mapping in beef processing reveals safety-relevant variations in microbial diversity and genomic features. *Food Research International* 186, 114318. <https://doi.org/10.1016/j.foodres.2024.114318>
97. SERIO, A., FUSELLA, G.C., PAPARELLA, A., 2011. SPOILAGE POTENTIAL OF H₂S PRODUCING BACTERIA IN SEAFOOD. *Italian Journal of Food Science / Rivista Italiana di Scienza degli Alimenti* 201–203.
98. Shahidi, F., 2016. Chapter 10 - Oxidative Stability and Shelf Life of Meat and Meat Products, in: Hu, M., Jacobsen, C. (Eds.), *Oxidative Stability and Shelf Life of Foods Containing Oils and Fats*. AOCS Press, pp. 373–389. <https://doi.org/10.1016/B978-1-63067-056-6.00010-0>
99. Shimizu, H., Iwamoto, S., 2022. Problems of Lipid Oxidation in Minced Meat Products for a Ready-made Meal during Cooking, Processing, and Storage. *Reviews in Agricultural Science* 10, 24–35. https://doi.org/10.7831/ras.10.0_24
100. Sobel, J., Lúquez, C., 2021. Chapter 24 - *Clostridium botulinum*, in: Morris, J.G., Vugia, D.J. (Eds.), *Foodborne Infections and Intoxications (Fifth Edition)*. Academic Press, pp. 405–416. <https://doi.org/10.1016/B978-0-12-819519-2.00001-3>
101. Srinivas, M., O’Sullivan, O., Cotter, P.D., Sinderen, D. van, Kenny, J.G., 2022. The Application of Metagenomics to Study Microbial Communities and Develop Desirable Traits in Fermented Foods. *Foods* 11, 3297. <https://doi.org/10.3390/foods11203297>

102. Stellato, G., La Storia, A., De Filippis, F., Borriello, G., Villani, F., Ercolini, D., 2016. Overlap of Spoilage-Associated Microbiota between Meat and the Meat Processing Environment in Small-Scale and Large-Scale Retail Distributions. *Applied and Environmental Microbiology* 82, 4045–4054. <https://doi.org/10.1128/AEM.00793-16>
103. Stellato, G., Utter, D.R., Voorhis, A., De Angelis, M., Eren, A.M., Ercolini, D., 2017. A Few *Pseudomonas* Oligotypes Dominate in the Meat and Dairy Processing Environment. *Front. Microbiol.* 8. <https://doi.org/10.3389/fmicb.2017.00264>
104. USDA, 2025. *Listeria Questions and Answers | Food Safety and Inspection Service [WWW Document]*. URL <http://www.fsis.usda.gov/food-safety/foodborne-illness-and-disease/illnesses-and-pathogens/listeria> (accessed 5.10.25).
105. Valdés, A., Álvarez-Rivera, G., Socas-Rodríguez, B., Herrero, M., Ibáñez, E., Cifuentes, A., 2022. Foodomics: Analytical Opportunities and Challenges. *Anal. Chem.* 94, 366–381. <https://doi.org/10.1021/acs.analchem.1c04678>
106. Valentino, V., De Filippis, F., Sequino, G., Ercolini, D., 2023. Psychrotrophic Bacteria Equipped with Virulence and Colonization Traits Populate the Ice Cream Manufacturing Environment. *Applied and Environmental Microbiology* 89, e00765-23. <https://doi.org/10.1128/aem.00765-23>
107. Valentino, V., Sequino, G., Cobo-Díaz, J.F., Álvarez-Ordóñez, A., De Filippis, F., Ercolini, D., 2022. Evidence of virulence and antibiotic resistance genes from the microbiome mapping in minimally processed vegetables producing facilities. *Food Research International* 162, 112202. <https://doi.org/10.1016/j.foodres.2022.112202>
108. Van Gompel, L., Luiken, R.E.C., Hansen, R.B., Munk, P., Bouwknecht, M., Heres, L., Greve, G.D., Scherpenisse, P., Jongerius-Gortemaker, B.G.M., Tersteeg-Zijderveld, M.H.G., García-Cobos, S., Dohmen, W., Dorado-García, A., Wagenaar, J.A., Urlings, B.A.P., Aarestrup, F.M., Mevius, D.J., Heederik, D.J.J., Schmitt, H., Bossers, A., Smit, L.A.M., 2020. Description and determinants of the faecal resistome and microbiome of farmers and slaughterhouse workers: A metagenome-wide cross-sectional study. *Environment International* 143, 105939. <https://doi.org/10.1016/j.envint.2020.105939>

-
109. Visciano, P., 2023. Good Hygiene Practice on Board Vessels for Fish Quality and Safety. <https://doi.org/doi:10.20944/preprints202308.2159.v2>
110. Wambui, J., Ghielmetti, G., Morach, M., Hochreutener, M., Stephan, R., 2021. Detection of Psychrophilic *Clostridium* spp. in Fecal Samples from Cattle of Different Ages Sampled at the Slaughterhouse Level. *Journal of Food Protection* 84, 58–62. <https://doi.org/10.4315/JFP-20-259>
111. Wang, G., Bei, S., Li, J., Bao, X., Zhang, Jiudong, Schultz, P.A., Li, H., Li, L., Zhang, F., Bever, J.D., Zhang, Junling, 2021. Soil microbial legacy drives crop diversity advantage: Linking ecological plant–soil feedback with agricultural intercropping. *Journal of Applied Ecology* 58, 496–506. <https://doi.org/10.1111/1365-2664.13802>
112. Wang, G., Wang, H., Han, Y., Xing, T., Ye, K., Xu, X., Zhou, G., 2017. Evaluation of the spoilage potential of bacteria isolated from chilled chicken *in vitro* and *in situ*. *Food Microbiology* 63, 139–146. <https://doi.org/10.1016/j.fm.2016.11.015>
113. Wang, J., Chen, J., Sun, Y., He, J., Zhou, C., Xia, Q., Dang, Y., Pan, D., Du, L., 2023. Ultraviolet-radiation technology for preservation of meat and meat products: Recent advances and future trends. *Food Control* 148, 109684. <https://doi.org/10.1016/j.foodcont.2023.109684>
114. Wang, X., Deng, Y., Sun, J., Ding, Y., Liu, Y., Tian, T., 2021. Unraveling characterizations of bacterial community and spoilage profiles shift in chilled pork during refrigerated storage. *Food Sci. Technol* 42, e80321. <https://doi.org/10.1590/fst.80321>
115. WHO, 2015. 1st Edition (2015) [WWW Document]. URL [https://www.who.int/teams/nutrition-and-food-safety/monitoring-nutritional-status-and-food-safety-and-events/foodborne-disease-estimates/1st-edition-\(2015\)](https://www.who.int/teams/nutrition-and-food-safety/monitoring-nutritional-status-and-food-safety-and-events/foodborne-disease-estimates/1st-edition-(2015)) (accessed 5.10.25).
116. Wickramasinghe, N.N., Ravensdale, J., Coorey, R., Dykes, G.A., Chandry, P.S., 2021. Transcriptional profiling of biofilms formed on chilled beef by psychrotrophic meat spoilage bacterium, *Pseudomonas fragi* 1793. *Biofilm* 3, 100045. <https://doi.org/10.1016/j.bioflm.2021.100045>

117. Xie, H., Guo, R., Zhong, H., Feng, Q., Lan, Z., Qin, B., Ward, K.J., Jackson, M.A., Xia, Y., Chen, X., Chen, B., Xia, H., Xu, C., Li, F., Xu, X., Al-Aama, J.Y., Yang, H., Wang, Jian, Kristiansen, K., Wang, Jun, Steves, C.J., Bell, J.T., Li, J., Spector, T.D., Jia, H., 2016. Shotgun Metagenomics of 250 Adult Twins Reveals Genetic and Environmental Impacts on the Gut Microbiome. *cells* 3, 572-584.e3. <https://doi.org/10.1016/j.cels.2016.10.004>
118. Xu, M.M., Kaur, M., Pillidge, C.J., Torley, P.J., 2023. Culture-dependent and Culture-independent Evaluation of the Effect of Protective Cultures on Spoilage-related Bacteria in Vacuum-packaged Beef Mince. *Food Bioprocess Technol* 16, 382–394. <https://doi.org/10.1007/s11947-022-02948-4>
119. Yang, X., Wu, Q., Zhang, J., Huang, J., Chen, L., Wu, S., Zeng, H., Wang, J., Chen, M., Wu, H., Gu, Q., Wei, X., 2019. Prevalence, Bacterial Load, and Antimicrobial Resistance of Salmonella Serovars Isolated From Retail Meat and Meat Products in China. *Front. Microbiol.* 10. <https://doi.org/10.3389/fmicb.2019.02121>
120. Yang, X., Youssef, M.K., Gill, C.O., Badoni, M., López-Campos, Ó., 2014. Effects of meat pH on growth of 11 species of psychrotolerant clostridia on vacuum packaged beef and blown pack spoilage of the product. *Food Microbiology* 39, 13–18. <https://doi.org/10.1016/j.fm.2013.10.008>
121. Yu, Y., Liang, Z., Liao, W., Ye, Z., Li, G., An, T., 2021. Contributions of meat waste decomposition to the abundance and diversity of pathogens and antibiotic-resistance genes in the atmosphere. *Science of The Total Environment* 784, 147128. <https://doi.org/10.1016/j.scitotenv.2021.147128>
122. Zdolec, N., 2017. *Fermented Meat Products: Health Aspects*. CRC Press.
123. Zhan, G., Pan, D., Zhou, C., Wang, Y., He, J., Zhang, J., Li, G., Cao, J., 2021. Characterizing bacterial strains of spoiled Jinhua ham and evaluating the effect of antimicrobial agents on these isolated bacterial strains. *LWT* 136, 110351. <https://doi.org/10.1016/j.lwt.2020.110351>
124. Zhang, B., Dou, H., Teng, S., Ye, K., 2025. *Pseudomonas fragi* and *Pseudomonas lundensis* drove the co-spoilage in chilled pork: Insights from the

- metabolome. Food Chemistry 464, 141717. <https://doi.org/10.1016/j.foodchem.2024.141717>
125. Zhang, H., Jain, C., Aluru, S., 2020. A comprehensive evaluation of long read error correction methods. BMC Genomics 21, 889. <https://doi.org/10.1186/s12864-020-07227-0>
126. Zhang, T., Zhang, W., Ai, D., Zhang, R., Lu, Q., Luo, Q., Shao, H., 2018. Prevalence and characterization of *Clostridium perfringens* in broiler chickens and retail chicken meat in central China. Anaerobe, MALDI-TOF MS in anaerobe diagnostics 54, 100–103. <https://doi.org/10.1016/j.anaerobe.2018.08.007>
127. Zhang, Y., Li, Q., Li, D., Liu, X., Luo, Y., 2015. Changes in the microbial communities of air-packaged and vacuum-packaged common carp (*Cyprinus carpio*) stored at 4 °C. Food Microbiology 52, 197–204. <https://doi.org/10.1016/j.fm.2015.08.003>
128. Zhao, F., Wei, Z., Zhou, G., Kristiansen, K., Wang, C., 2022. Effects of Different Storage Temperatures on Bacterial Communities and Functional Potential in Pork Meat. Foods 11, 2307. <https://doi.org/10.3390/foods11152307>
129. Zhou, C., Zhan, G., Pan, D., Zhou, G., Wang, Y., He, J., Cao, J., 2022. Charactering the spoilage mechanism of “three sticks” of Jinhua ham. Food Science and Human Wellness 11, 1322–1330. <https://doi.org/10.1016/j.fshw.2022.04.021>
130. Zhou, C.-Y., Pan, D.-D., Cao, J.-X., Zhou, G.-H., 2021. A comprehensive review on molecular mechanism of defective dry-cured ham with excessive pastiness, adhesiveness, and bitterness by proteomics insights. Comprehensive Reviews in Food Science and Food Safety 20, 3838–3857. <https://doi.org/10.1111/1541-4337.12779>
131. Zhu, Y., Gu, M., Su, Y., Li, Z., Xiao, Z., Lu, F., Han, C., 2024. Recent advances in spoilage mechanisms and preservation technologies in beef quality: A review. Meat Science 213, 109481. <https://doi.org/10.1016/j.meatsci.2024.109481>
132. Zhu, Y., Wang, W., Li, M., Zhang, J., Ji, L., Zhao, Z., Zhang, R., Cai, D., Chen, L., 2022a. Microbial diversity of meat products under spoilage and its controlling approaches. Front. Nutr. 9. <https://doi.org/10.3389/fnut.2022.1078201>

133. Zhu, Y., Wang, W., Zhang, Y., Li, M., Zhang, J., Ji, L., Zhao, Z., Zhang, R., Chen, L., 2022b. Characterization of Quality Properties in Spoiled Mianning Ham. *Foods* 11, 1713. <https://doi.org/10.3390/foods11121713>

CHAPTER 3

Mapping the Microbiome along Beef Processing Chain Highlights the Impact of Seasonal Variations

Highlights:

- A “seasonal inversion” was discovered: microbial diversity peaked in summer on processing surfaces but in winter on final meat products.
- Antibiotic resistance (AMR) gene potential peaked in winter, linked to indoor animal housing, while most other functional risks peaked in summer, linked to outdoor animal rearing.
- Routine sanitation failed to reset the house microbiome, paradoxically enriching biofilm, and biocide resistance genes by up to 3.2-fold.
- Long, undisturbed maturation selected for slow-growing specialists like *Carnobacterium*.
- Disturbance-driven retail maturation triggered an inverted succession, favouring fast-growing opportunists like *Lactococcus*.
- Bacteriophages play a dual role: widespread “peaceful” coexistence, but also localized “battles” where they suppress *Listeria* and *Salmonella*.
- Maturation confirmed a key trade-off: pathogen signals were reduced to non-viable levels (no MAGs), while spoilage-causing bacteria were highly enriched.

Abstract

Meat-processing environments harbour complex microbial ecosystems that influence product quality and safety. However, the combined effects of season, sanitation procedures, and bacteriophage activity on these ecosystems remain unclear. To address this, we conducted a longitudinal farm-to-fork investigation across four beef processing facilities over two seasons using shotgun metagenomics. A total of 712 samples were collected during the production and post-cleaning stages, followed by industrial wet-aging (2-77 days) and the retail shelf-life period (0-24 days). This approach has revealed distinct ecological patterns. Indeed, long-term maturation was dominated by *Carnobacterium*, whereas *Lactococcus* species dominated during the retail period. Furthermore, environmental microbiomes exhibited broad metabolic potential, in contrast to the specialized, low-diversity profiles of mature meat. Routine sanitation failed to eliminate the resident microbial communities, although it exerted selective pressure, reducing spoilage taxa while enriching biofilm-forming genes by up to 3.2-fold in post-cleaning samples. Additionally, bacteriophages showed a positive overall association with their bacterial hosts; however, niche-specific analyses revealed strong antagonistic effects against *Listeria monocytogenes* and *Salmonella enterica*. Collectively, these findings demonstrate that beef processing microbiomes are structured by the interaction of multiple ecological forces rather than by sanitation alone. Understanding these interactions provides a comprehensive framework for ecology-based strategies to improve meat quality, safety, and shelf life.

Keywords: *Microbiome Ecology, Bacteriophage-Host Interactions, Functional Microbiome Profiling, Sanitation Resilience*

1. Introduction

Globally, approximately one quarter of all meat is lost at the production or consumer level, resulting in significant food waste and revenue losses (Karwowska et al., 2021). This is further inflated by a substantial environmental footprint, due to the use of water, feed, energy, and increased methane emissions associated with both animal farming and wasted meat (World Wildlife Fund, 2021; Poore and Nemecek, 2018; FAO, 2013). For instance, in the United States, an estimated 194 million kilograms (kg) of meat is discarded annually due to spoilage, which is equivalent to the waste of up to 780,000 animals and their associated resources used in their production (Ramanathan et al., 2022). These losses highlight a disturbing reality: understanding and controlling microbial dynamics in commercial meat processing is not merely a matter of quality control, but also a fundamental challenge for global food security and environmental sustainability.

The meat-processing environment leads meat spoilage. These facilities harbour resilient house microbiomes that live in complex ecosystems, particularly in biofilms offering protection against rigorous sanitation procedures (De Filippis et al., 2021), that can end up on carcasses after processing. Previous studies have highlighted that these resident communities are a rich source of spoilage (e.g., *Pseudomonas*, *Acinetobacter*, *Psychrobacter*, *Brochothrix*, and *Moraxella*) and pathogenic microorganisms (e.g., *Salmonella enterica* and *Listeria monocytogenes*) (De Filippis et al., 2021; Véghová et al., 2017; Yeom et al., 2024) showing a wide array of functional risks, including biocide resistance, spoilage, virulence, and antimicrobial resistance genes (AMR; (Barcenilla et al., 2024; Cobo-Díaz et al., 2021; Sequino et al., 2025). In addition to the meat industry, this problem is also well known in other food industries, such as dairy (De Filippis et al., 2024), vegetables (Valentino et al., 2022), and ice cream (Valentino et al., 2023). Hence, the current literature firmly establishes that the food-processing environment is not simply a sedentary location but an active source of contamination that profoundly shapes the microbial structure of food products from the very beginning.

Despite improvements in sequencing technologies and hygiene monitoring, the current understanding of microbial dynamics in modern meat-processing chains remains fragmented. Most studies have assessed these processes either by focusing on single processing facilities in limited timeframes or by examining processing chains and matured meat products in isolation. Some studies have explored only the physicochemical or sensory attributes of long-term wet

aging. Consequently, the critical transitions that shape microbial progression from initial contamination during processing to stable communities in mature products remain poorly understood. Furthermore, the potential contribution of bacteriophages as ecological drivers shaping the meat microbiome has rarely been explored.

Therefore, to bridge these knowledge gaps, this study employed a comprehensive farm-to-fork longitudinal shotgun metagenomic survey across four commercial beef processing facilities in summer and winter seasons. This unique design allowed us to explore several dimensions together for the first time: (1) a comparative view of the beef microbiome structure and function in two contrasting seasons, (2) a comprehensive story of microbial succession along the processing chains through long-term wet aging up to the short-term retail period, and (3) the integrative analyses of sanitation efficacy and bacteriophage-host interplay in determining the microbial outcome of the final product. These explorations provide a comprehensive ecosystem-level understanding of how factors such as season, sanitation, and bacteriophages collectively interact to define the safety, quality, and longevity of the beef products.

2. Methodology

2.1.1. Sampling Strategy

Four Irish beef processing facilities were visited during summer 2023 and winter 2024. Food contact and non-food contact samples were collected from the farm, slaughter hall, chiller hall, boning hall and the retail hall both during processing and after sanitation by swabbing with Whirl-Pak Hydrated PolyProbe swabs (Whirl-Pak, Madison, Wisconsin, US), covering 1 m² for points with undefined area or one unit for smaller items (e.g., knives; Supplementary Table 1 and Figure 1). All personnel wore protective coats, shoes, caps, and facial masks throughout the sampling, and changed gloves between samples to prevent cross-contamination. All samples collected from a specified processing location, such as a slaughter hall, were combined, placed in a single crate, and immediately transferred to the company refrigerator.

In addition, both left and right silverside of carcasses were sampled as described above. Furthermore, the final meat products underwent controlled wet-aging, followed by refrigerated storage during the retail shelf-life phase. Vacuum-packed products were kept at 0–3 °C in a cooling chamber. Long-term "Continuous maturation" involved the maturation of meat chunks for 2, 10, 22, 35, 49, and 77 days. While retail phase involved taking the previously matured meat (but only the 22-day-old meat as mentioned above), cutting it into retail portions. Samples from the retail were collected at baseline and after 3, 10, 17, and 24 days of storage.

However, 28 samples were excluded from sequencing due to logistical issues or insufficient DNA yield.

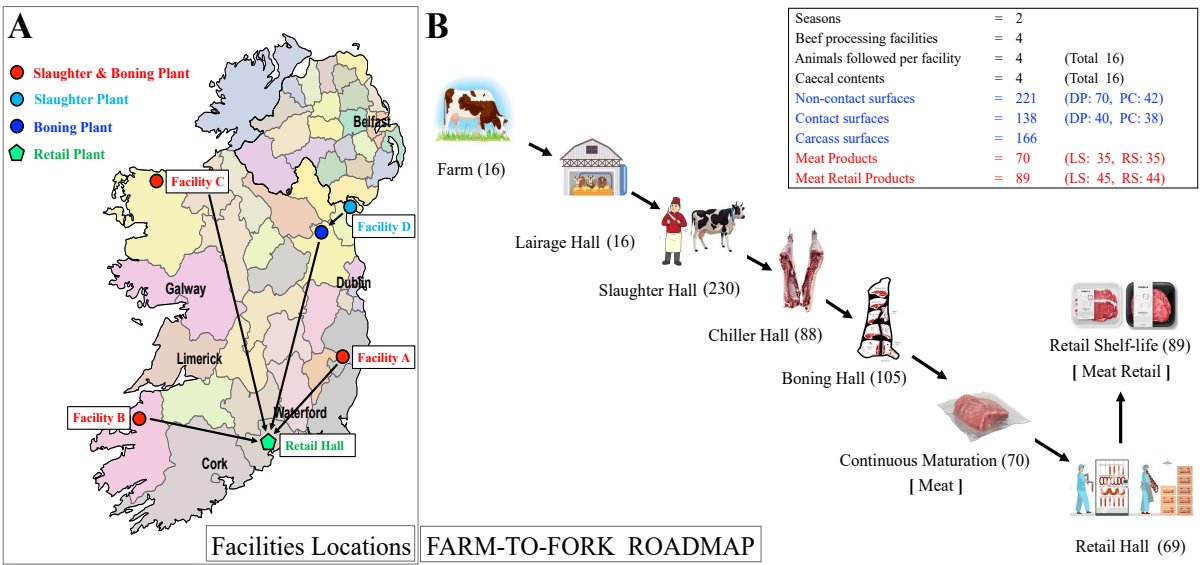


Figure 1: Sampling locations and strategy. (A) Map of the selected facilities across the Republic of Ireland. Different colours represent different processing stages at each location. The Slaughter and Chiller Hall are always at the same site. **(B)** A detailed workflow of the sampling points i.e., from farm-to-fork. Numbers in brackets represent the total sample count from that specific processing step. Abbreviations in the table: DP stands for During Production, PC for Post Cleaning, LS for Left Silverside, and RS for Right Silverside. The map is created using “rnatualearth”, and “rnatualearthdata” R packages.

2.1.2. DNA Extraction and Sequencing

All swabs were packed and shipped in refrigerated trucks to the laboratory for further processing. Microbial cells were recovered from each swab in sterile phosphate-buffered saline solution supplemented with 0.1% (v/v) Triton X-100 (PBS-T; 137 mM NaCl, 10 mM Na₂HPO₄, 1.8 mM KH₂PO₄, pH 7.4). The cells were detached from the swabs using a homogenizer (300 rpm, 90 s), and the suspension was centrifuged at $12,000 \times g$ for 2 min. This process was repeated to maximize cell recovery. The resulting pellet was washed twice with sterile PBS and stored at -80°C until DNA extraction.

DNA was extracted from the stored pellets using the MoLYsis™ Complete5 Kit (Molzym, Bremen, Germany) according to the manufacturer's instructions. DNA concentrations were quantified using a Qubit HS Assay (Thermo Fisher Scientific, Waltham, MA, USA). Sequencing libraries were prepared with the Nextera XT Index Kit v2 (Illumina, San Diego, CA, USA). Whole-metagenome shotgun sequencing was performed on an Illumina NovaSeq platform to generate 2×150 bp paired-end reads.

Shotgun metagenomic sequencing generated a total of 21.72 billion raw reads across two batches collected during summer 2023 (11.23 billion reads; 33.44M avg. per sample) and winter 2024 (10.49 billion reads; 30.22M avg. per sample).

2.2. Bioinformatic Analysis

2.2.1. Raw Sequencing Data Transformation into Clean Processed Data

Host paired-end reads from each sample were removed by mapping to the *Bos taurus* reference genome (NCBI Accession PRJNA391427) using the Best Match Tagger (https://www.hmpdacc.org/hmp/doc/HumanSequenceRemoval_SOP.pdf), with default

options. The remaining non-host microbial reads were quality-filtered using Prinseq-lite (v0.20.4) to trim low-quality reads from the 3' ends (*-trim_qual_right 5*) and remove reads shorter than 60 bp (*-min_len 60*) (Schmieder and Edwards, 2011). After quality filtering of the raw reads, samples with significant read loss were excluded from the analysis. The number of raw and filtered reads remaining for all samples are reported in Supplementary Table 1.

Taxonomic profiling was performed on the clean filtered reads using Kraken2 (v2.0.8-beta, with default settings; Wood et al., 2019) build with the PlusPF database including the *Bos taurus* genome (to effectively remove any remaining host-associated reads), updated on January 12, 2024 (Langmead, n.d.). Bracken (v2.5; *-r 150, -l 5, -t 40*) was then applied to refine taxonomic assignments and estimate species-level abundances (Lu et al., 2017), using the same indexes built from the Kraken2 database.

High-quality reads from each sample were independently assembled into contigs using MEGAHIT (v1.2.2; *--k-list 21,33,55,71,81,91*; Li et al., 2015), and contigs < 1,000 bp were discarded. Filtered reads were mapped to their respective sample's contigs using Bowtie2 (v2.2.9) with "*--very-sensitive-local*" and "*--no-unal*" options (Langmead and Salzberg, 2012). Contigs and the files resulting from mapping were processed with MetaBAT2 (v2.12.1; *-m 1500 --unbinned*; Kang et al., 2019) to group similar contigs into bins, and the quality of bins was assessed using CheckM2 (v1.0.1; Chklovski et al., 2023). MAGs showing completeness $\geq 50\%$ and contamination $\leq 10\%$ ("medium quality" MAGs, according to Benoit et al., 2024) were retained for further analysis.

Finally, the taxonomy was assigned to each representative SGB using the (*classify_wf*) parameter of the Genome Taxonomy Database Toolkit (GTDB-Tk v2.4.0) with the release 220 database, and MAGs were placed into a standardized, robust phylogenetic tree-based SGBs on a suite of single-copy marker genes (Chaumeil et al., 2020).

2.2.2. Functional and Viral Annotation of the Assembled Contigs

The functional potential of the assembled contigs was characterized using homology searches against several curated databases. For all protein-level searches, a predicted coding sequence (CDS) was considered a significant hit only if the alignment covered $\geq 90\%$ of the reference gene length showing $\geq 90\%$ gene identity.

AMR and biocide-resistance genes were identified by searching the predicted CDSs against the Comprehensive Antibiotic Resistance Database (CARD v3.2.4) protein homolog and the BacMet2 database (Pal et al., 2014), respectively, using BLASTx (Alcock et al., 2023). Virulence factor genes were detected using BLASTn searches against the core nucleotide dataset of the Virulence Factor Database (VFDB, 2022 release) (Liu et al., 2022). Finally, to identify spoilage-related genes, an extensive literature search was conducted to construct a custom protein database focusing on meat spoilage. This database comprises 336 curated spoilage-related genes involved in key pathways, including protein metabolism, lipid metabolism, sulphur metabolism, regulatory pathways, siderophore production, biofilm formation, stress adaptation, and quorum sensing. To enhance the detection sensitivity, this core set of genes was expanded with 4,653 orthologous sequences sourced from a diverse range of bacterial species in the NCBI protein database. The custom spoilage gene database, which includes a complete description of all the included genes is publicly available online ([DOI: 10.5281/zenodo.17494165](https://doi.org/10.5281/zenodo.17494165)).

To accurately assign taxonomy to the assembled contigs from each sample, which were binned earlier into MAGs.

To link functional potential with microbial taxa, taxonomy was assigned to the assembled contigs based on their parent MAGs. Since MAGs were taxonomically classified using GTDB-Tk, a mapping file was generated that passed down this species-level identity for its every constituent contig. Consequently, this file containing the sample ID, contig ID, and species-level information was used to produce a statistically robust measure of functional gene abundance. The quantification strategy developed in this manner corrects for both gene length bias and sequencing depth. This normalized abundance was then aggregated at two complementary scales: the overall community level and the species-specific level.

First, to quantify the overall functional potential of the entire microbial community within a sample, the Community-Level Functional Load was calculated. The numerator was derived by summing the weighted abundances of all significant functional gene hits in a sample. Then, the weighted abundance for each hit was calculated as its parent contig coverage (the "*multi*=" value from the assembly header) divided by the full length of the reference gene in kilobases (kb). This sum was then normalized by the total number of bacterial reads in that sample (in millions), as determined from the Bracken output "tab file" of the filtered reads. The results

were presented in units termed "functional score." The higher the score, the higher the functional load within that sample or taxon. The formula is as follows.

$$\text{Community – Level Functional Load} = \frac{\sum_{\text{all gene hits in sample}} \left(\frac{\text{Contig Coverage}}{\text{Gene length in kb}} \right)}{\text{Total Bacterial Reads in that Sample (in Millions)}}$$

Likewise, to attribute this functional potential to specific taxa and to identify key reservoir species, a Species-Level Functional Load was calculated. The process was similar, but the numerator was first calculated per species by summing the weighted abundances of only those gene hits found in contigs taxonomically assigned to that species via GTDB-Tk. This species-specific score was then normalized by the total number of reads explicitly assigned to that species by Bracken (in millions), yielding a measure of the functional density within that species' genome. The formula is as follows.

$$\text{Species – Level Functional Load} = \frac{\sum_{\text{all gene hits in Species}} \left(\frac{\text{Contig Coverage}}{\text{Gene length in kb}} \right)}{\text{Total Reads for that species (in Millions)}}$$

The assembled contigs from each sample were analysed to identify bacteriophages using the end-to-end workflow of PhaBOX2 (v2.1.11) against the phabox_db_v2 internal database. This resulted in predictions for the viral taxonomy and lifestyles (lytic or temperate), along with prediction scores and confidence values, and potential bacterial hosts (Shang et al., 2023). The assigned viral contigs from PhaBOX2 were quantified to obtain abundance values using Bowtie2 (v2.2.9) by mapping the raw reads from each sample back to their predicted phage contigs. Hence, the resulting phage read counts were used to generate an abundance matrix of phages across all the samples.

2.3. Statistical Analysis and Visualizations

All statistical analyses were conducted using R (v4.3.3) (<https://cran.r-project.org/>). Alpha diversity indices (Shannon, Simpson, and Observed Richness) were calculated for each sample using the vegan package in R (vegan, n.d.). The results were visualized as boxplots using the ggplot2 package, with individual data points superimposed using the ggbeeswarm package (ggplot2, n.d.). Significance values were added to these plots using the compare_means function in the ggpubr package, through the Kruskal-Wallis and Pairwise Wilcoxon rank-sum tests. Statistical significance was set at $p < 0.05$, and all p-values were also adjusted through

the Benjamini and Hochberg using the (BH) argument in the `compare_means` function from the `ggpubr` package. For the taxonomic level stacked bar charts and heatmaps, the `ggplot2` and `pheatmap` packages were used, while the phylogenomic tree was developed using the `ggtree` package.

Beta diversity was assessed by computing a pairwise dissimilarity distance matrix based on the Bray-Curtis distance through the function `'vegdist'` from the `vegan` R package. A principal component analysis (PCoA) was then performed on this matrix through the `'cmdscale'` function from the `'base'` R package. Similarly, the effects of the metadata variables (i.e., seasons, locations and surfaces) on the overall community composition were tested using a permutational multivariate analysis of variance (PERMANOVA) with the `'adonis2'` function (999 permutations). Furthermore, we computed the intra-group dispersion homogeneity using the `'betadisper'` function from the `'vegan'` package.

Among those taxa detected in at least 90 % of the samples from a niche (e.g., summer slaughter hall), we defined niche-specific core microbiome composed of species showing a relative abundance $\geq 1\%$ in $\geq 20\%$ of the samples from that niche (Barcenilla et al., 2024).

Furthermore, to correlate bacteriophage abundance with their targeted bacterial hosts, linear mixed-effects models were implemented using the `lme4` and `lmerTest` packages in R, considering bacterial host abundance, phage abundance, scaled sample load (i.e., sequencing depth) and season as fixed effects, and processing location, host and other metadata as random effects. The final model was selected among approximately 35 candidate models based on a combination of model parsimony, lowest Akaike Information Criterion (AIC), and Bayesian Information Criterion (BIC). The selected model structure was as follows: `lmer(log_Host_Abundance ~ log_Phage_Abundance × Scaled_Load + (1 | major_location) + (1 | Host))`. The same model was employed at the location and individual sample-point levels. Sample point-level model results for key meat-relevant species that showed statistically significant suppression by their associated bacteriophages were visualized using forest plots, with positive associations excluded for clarity.

2.5. Data availability: The raw shotgun metagenomic sequencing data generated in this study are publicly available in the NCBI Sequence Read Archive (SRA) under BioProject accession number PRJNA1233666.

3. Results:

3.1. Meat Processing Environment Acts as an Ecological Filter by Gradually Reducing the Environmental Microbiome to a Focused Microbiome on the Final Meat Products

The Principal Coordinate Analysis (PCoA; only during production samples) based on Bray–Curtis dissimilarities explained 31.7% of the variance on the primary two axes, and displayed two distinct clusters mainly driven by the surface type (i.e.; industrial surfaces or meat products; PERMANOVA p -value < 0.001). The first cluster comprised the processing environmental surfaces (i.e., non-contact, contact) and carcasses, while the second was tightly aggregated and consisted only of the Meat and Meat Retail Products (Figure 2A). Additionally, the experimental groups, especially the environmental surfaces also showed a significant intra-group dispersion (p -value < 0.001) (Figure 2A & Supplementary Figure 1A).

Interestingly, we observed a consistent reduction of microbial diversity indices throughout the process across all facilities and seasons (Figure 2B). Indeed, the highest species richness was observed in the farming environment (i.e., Farm summer: $8,926 \pm 863$,; winter: $8,193 \pm 3,838$,; Lairage hall summer: $9,980 \pm 418$,; winter: $10,084 \pm 591$) and the lowest in the retail hall (Retail hall summer: $1,537 \pm 2,037$,; winter: $2,017 \pm 2,258$,; Supplementary Figure 1B). This progressive reduction was observed in both food contact and non-food contact surfaces across the processing locations even regardless of season (Supplementary Figure 1C–D). No significant differences were observed in richness between summer and winter samples, except for the slaughter hall ($p < 0.05$), meat ($p < 0.01$), and meat retail products ($p < 0.001$; Figure 2B). A similar progressive reduction in richness was observed between early and late maturation stages in meat products, although differences were not significant at any individual maturation time points. Interestingly, a significant temporal shift in richness between early and late maturation stages was detected only during winter, but not in summer (Supplementary Figure 1E–F).

Even though the environmental species richness decreased over the process, the Simpson's evenness index remained largely stable, indicating that no single taxon disproportionately dominated the community (Figure 2B). The only exception was the contact and carcass surfaces from the retail hall, where a more selected microbial community was observed (Figure 2B). Furthermore, as expected, the microbial diversity of meat decreased during maturation in both the facility and the retail phase (Supplementary Figure 1E–F). Ultimately, the core

microbial community profile was highly resilient to sanitation interventions, as no significant differences were observed between samples collected during active production and post-cleaning at any processing stage (Supplementary Figure 1G), nor between left and right-sided silversides in either season.

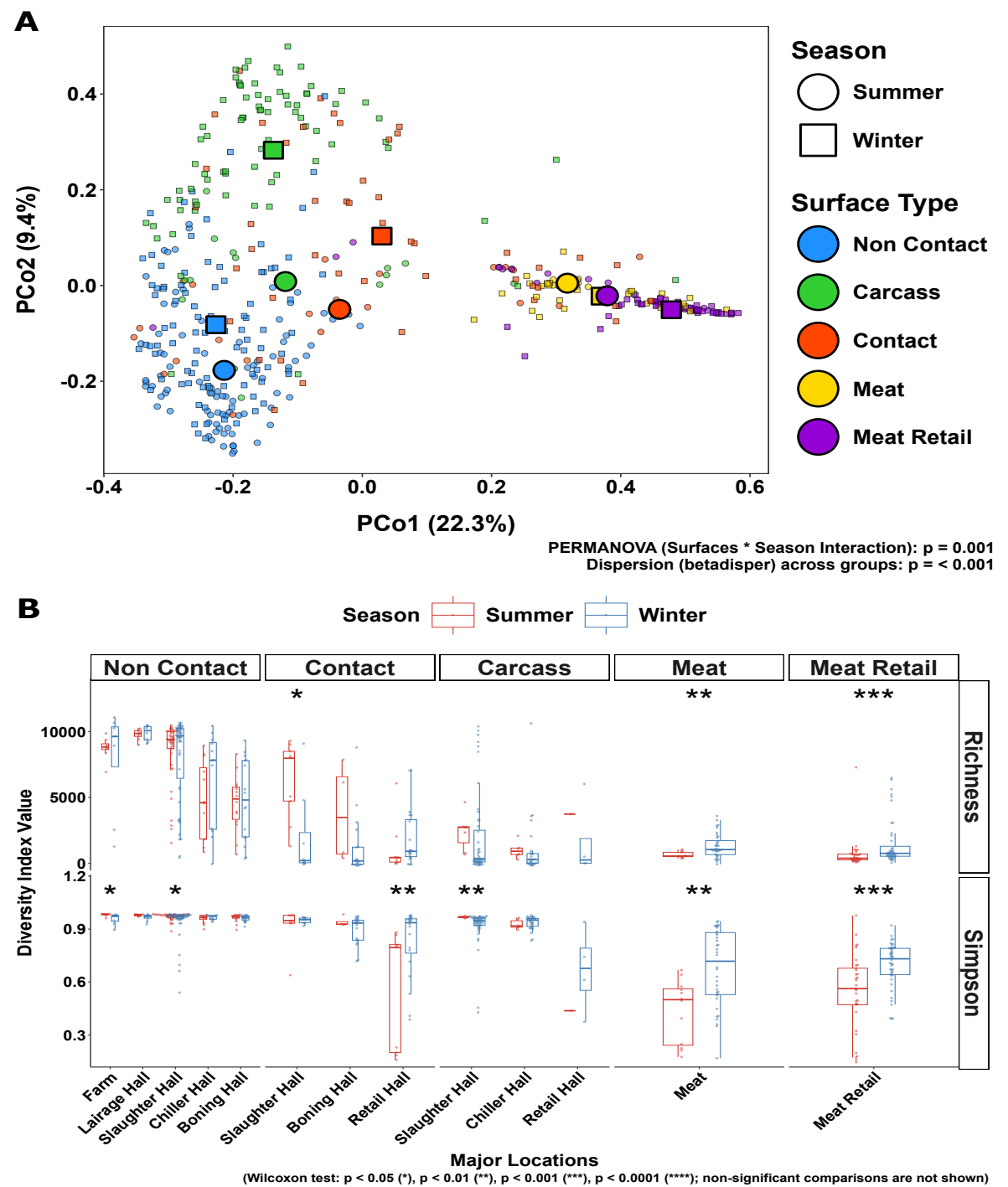


Figure 2: Alpha and Beta Diversity. (A) PCoA ordination plot (using Bray-Curtis Dissimilarity) of the processing surfaces and final meat products. Colours represent different

surfaces/products, while shapes (circles and squares) represent the season. **(B)** Seasonal comparisons of alpha diversity (Richness and Simpson Index) for the processing surfaces at each location, and final meat products. Boxplots were compared using the Wilcoxon Rank-Sum test. Asterisks indicate significance: $p < 0.05$ (*); $p < 0.01$ (**); $p < 0.001$ (***); $p < 0.0001$ (****). “ns” means not significant (most non-significant comparisons are not shown).

3.2. Taxonomic Composition Revealed a Seasonally Driven Core Microbiome from Processing to the Final Meat Products

3.2.1. Processing Locations Drive a Taxonomic Shift from the Farm Associated Diversity to a Lactic Acid Bacteria Dominated Retail Hall

The meat processing environment showed a seasonally dynamic taxonomic composition, with a clear ecological progression from a diverse farm-associated microbial community to a more specialized LAB-dominated community in the final processing stages. The winter core community was dominated by *Bifidobacterium pseudolongum* and *Cutibacterium acnes* in many locations as compared to the more diverse array of microbial species, including *Acinetobacter johnsonii* and *Corynebacterium xerosis* in summer (Supplementary Figure 2A-D).

Beyond these seasonal core patterns, microbial community composition differed significantly among the processing locations, independent of season (Supplementary Table 2). To further resolve location-associated ecological signatures, log₂ fold-change relative to the global mean was used to identify hall-enriched taxa and their seasonal shifts (Supplementary Figures 3–4). Together, these analyses reveal distinct, location-specific microbial signatures superimposed on a seasonally structured core microbiome across the processing environment.

Interestingly, the operator's hands and knives might act as key vectors for meat-associated spoilage bacteria, particularly in the Boning and Retail Halls, as we observed high mean relative abundances of *Lactococcus carnosus* (34.1%) and *Dellagليا algida* (16.2%) on operator's hands sampled in winter. Consistently, *D. algida* dominated on knives (15.2%), and primals at debagging (20.2%) in the Retail Hall (Supplementary Figure 5A). Conversely, these same sample points in Summer were dominated by *Latilactobacillus curvatus* on operator hands (44.3%), knives (28.2%), and primals (73.9%) (Supplementary Figure 5A). Operators might also transfer pathogenic microorganisms, as *Salmonella enterica* accounted for ~9.1 %

of the microbiome on operators' hands on average, and ~9.1 % on knives (Supplementary Figure 5C). Similarly, vacuum bags harboured a plethora of opportunistic pathogens, including *E. faecium*, *A. baumannii*, *S. aureus*, *Pseudomonas aeruginosa*, and *Enterobacter hormaechei*, with a total mean relative abundance of 13% in winter and 2.2% in summer (Supplementary Figure 5C).

3.2.2. Sanitation Efforts Restructure the Taxonomic Composition of the Environmental Microbiome

The abundance of some species was not affected by sanitation. Indeed, *A. johnsonii* persisted across all locations and seasons. Conversely, sanitation effectively suppressed dominant spoilage organisms in Retail Hall, such as *L. curvatus*, which decreased from 54.0% to 4.12%, and *Carnobacterium divergens* dropped from 14.0% to undetectable levels in summer. Similarly, the dominant winter species, *D. algida*, showed higher resistance and declined only from 23.2% to 3.85% (Supplementary Figure 5B).

The pathogenic taxa showed variable responses depending on season and processing location. However, *E. faecium*, *A. baumannii*, *K. pneumoniae*, *P. aeruginosa*, *S. enterica*, *E. coli*, and *S. aureus* were found to be more resilient across locations and seasons. This suggests that while sanitation suppresses many spoilage bacteria, it may simultaneously enrich for persistent and stress-tolerant pathogens (Supplementary Figure 5D).

3.2.3. Surface Types and Maturation Protocols Drive Microbiome Division into Environmental Generalists and Final Product Specialists

A general trend of microbial succession from a taxonomically diverse environmental community into a specialized LAB-dominated profile in meat samples has been observed in both seasons. As expected, non-contact surfaces showed a diverse community with low-abundant spoilage taxa in both seasons, while contact and carcass surfaces were co-dominated by LAB consortia (*Latilactobacillus*, *Carnobacterium*, and *Lactococcus*). This was seasonally dependent: summer contact and carcass surfaces were dominated by *L. curvatus*, whereas *D. algida* predominated in winter (Figure 3A).

On the contrary, the microbial community in meat samples was less variable and mainly not influenced by seasons, with a few exceptions. In detail, during long-term continuous maturation

in summer, *C. divergens* maintained stable dominance (61–63%), while *C. maltaromaticum* was steadily displaced (31.6% → 11.9%) by *L. curvatus* (2.9% → 24.3%) over time (Meat 35 → Meat 77). In winter, a diverse initial community transitioned to co-dominance by *L. carnosus* (31.8%) and *C. divergens* (40.6%), while *L. paracarnosus* declined sharply (23.2% → 0.5%). Low-abundance species such as *Leuconostoc gelidum*, *L. sakei*, and *L. piscium* persisted throughout the continuous maturation (Figure 3B).

Conversely, the retail maturation process progressed more rapidly. In summer, an initial dominance of *L. curvatus* (60.2%) was replaced by *L. sakei* (86%) by day 3, which was then substituted by *L. carnosus* (55.6%) and *L. paracarnosus* (19.1%) by the end of shelf life. In winter, the early stages (day 0–3) were co-dominated by *Latilactobacillus*, *Lactococcus*, and *Carnobacterium*, transitioning to a dominance by *L. paracarnosus* (54.6%) and *L. carnosus* (21.3%) at day 24. Overall, spoilage microbiome development followed a time, temperature, and protocol-dependent trajectory: continuous maturation favoured stable dominance by a few key taxa, whereas retail maturation supported faster, more dynamic successions shaped by season (Figure 3B). Notably, several well-known meat spoilage taxa, including *Pseudomonas fragi*, *P. fluorescens*, *Aeromonas hydrophila*, *Serratia liquefaciens*, *S. proteamaculans*, *Proteus mirabilis*, *Pantoea agglomerans*, *Shewanella putrefaciens*, and *Weissella viridescens*, were also screened but were either sporadically detected or remained at very low abundances (<0.5% mean relative abundance) across all experimental groups.

Parallel analysis of the foodborne pathogens revealed persistent but low-level occurrence across environmental surfaces and seasons. *E. faecium*, *A. baumannii*, *K. pneumoniae*, *C. perfringens*, *S. aureus*, *S. enterica*, and *L. monocytogenes* were detected across all surface types in both seasons, generally below 2% mean relative abundance (Figure 3C). In both maturation protocols, their mean relative abundances dropped further (<0.2%) across all time points. However, the only prominent exception was the isolated detection of *K. pneumoniae* (12.1%) and *Yersinia enterocolitica* (8.4%) in a single retail sample at day 0 in summer. But as maturation progressed, these species declined sharply (Figure 3D).

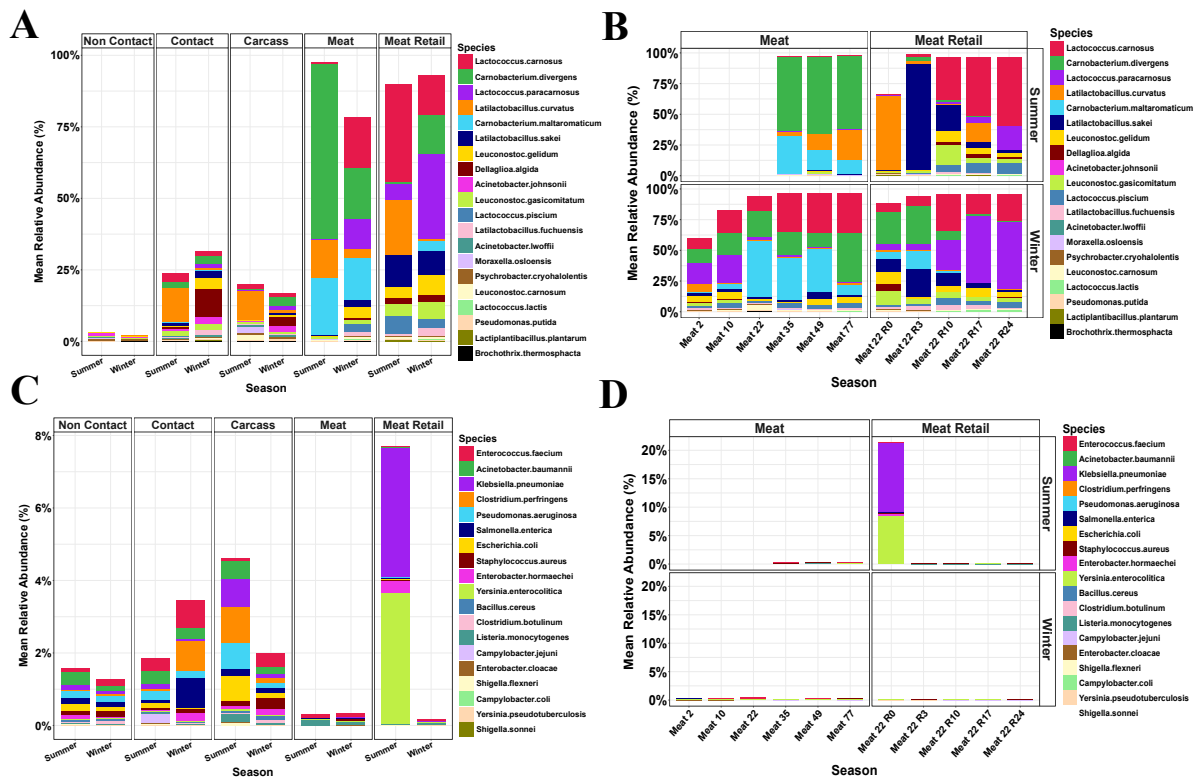


Figure 3: Abundance of meat-relevant spoilage and pathogenic bacteria across season. (A) Mean relative abundance (%) of spoilage species on processing surfaces and in final meat products. Species are sorted by abundance in the whole dataset for each plot (B) Mean relative abundance (%) of spoilage species in matured meat products at each specific maturation timepoint. (C) Mean relative abundance (%) of pathogenic species on processing surfaces and in final meat products. (D) Mean relative abundance (%) of pathogenic species in matured meat products at each specific maturation timepoint.

3.3. The Microbial Functions were Restructured Along the Processing Chain, and were Dramatically Shifted from a Diverse Environmental Reservoir to a Negligible and Distinct Functional Profile on the Final Meat Products

We then estimated the prevalence and the abundance of metabolic activities negatively impacting on food quality and safety and securing microbial communities against biotic/abiotic stresses. Overall, the abundance of spoilage genes at processing locations was higher in summer than winter, and summer carcasses showed the highest CPM value (Wilcoxon's rank sum test p -value < 0.001 ; Figure 4A). This overall load was more pronounced in the summer Retail Hall (median = 640), followed by the summer Chiller Hall (Supplementary Figure 6A).

Among spoilage-associated genes, those involved in biofilm formation were the most abundant across most environmental surfaces and locations, particularly in Chiller, Boning and Retail Halls during summer (Supplementary Fig. 6B), and were mainly encoded by *L. paracarnosus* and *D. alga* (Supplementary Fig. 6C). Furthermore, biofilm-associated gene load was found higher in post-cleaning samples (mean CPM = 820) compared to during production (mean CPM = 257), corresponding to an average 3.2-fold increase, although not significant (Wilcoxon rank-sum test, $p = 0.38$; Supplementary Fig. 6D). This diverse environmental spoilage potential transitioned to a specialized one in the final meat products but was maturation-dependent. Continuous maturation showed a spike in regulatory pathways in both seasons largely attributed to *Carnobacterium* species. At the same time, retail maturation showed high abundance of quorum sensing genes and were mainly attributed to *L. carnosus*, while biofilm formation was observed in both seasons (Supplementary Fig. 6C).

On the contrary, genes conferring resistance to different classes of antimicrobial compounds were generally low, and their abundance was not influenced by the season, except for carcasses from the summer sampling, where antimicrobial resistance was enhanced compared to the winter counterpart (Figure 4C-D). The most relevant genes encoded resistance against macrolides, especially on contact surfaces in the winter slaughter hall, and were mainly attributed to *Brachybacterium conglomeratum*, while tetracycline resistance genes that increased in meat retail samples and were mostly detected in *L. paracarnosus* and *C. maltaromaticum*, respectively (Figure 4C and Supplementary Fig. 7A-F).

Finally, virulence and biocide resistance genes were detected at very low abundances across the processing environment and meat products and were predominantly associated with the environmental microbiome (Supplementary Figures 8A-F and 9A-F). In parallel, several taxa displayed niche-specific localization along the processing chain; for example, *Klebsiella grimontii*, *Stenotrophomonas indicatrix*, *Enterobacter ludwigii*, and *Aeromonas hydrophila* were primarily detected on carcass surfaces during summer. In contrast, other species were widely distributed across processing surfaces. Notably, *Enterococcus faecalis* was consistently detected throughout the production chains in both seasons and carried a substantial functional load across nearly all metabolic pathways examined (Figure 4E).

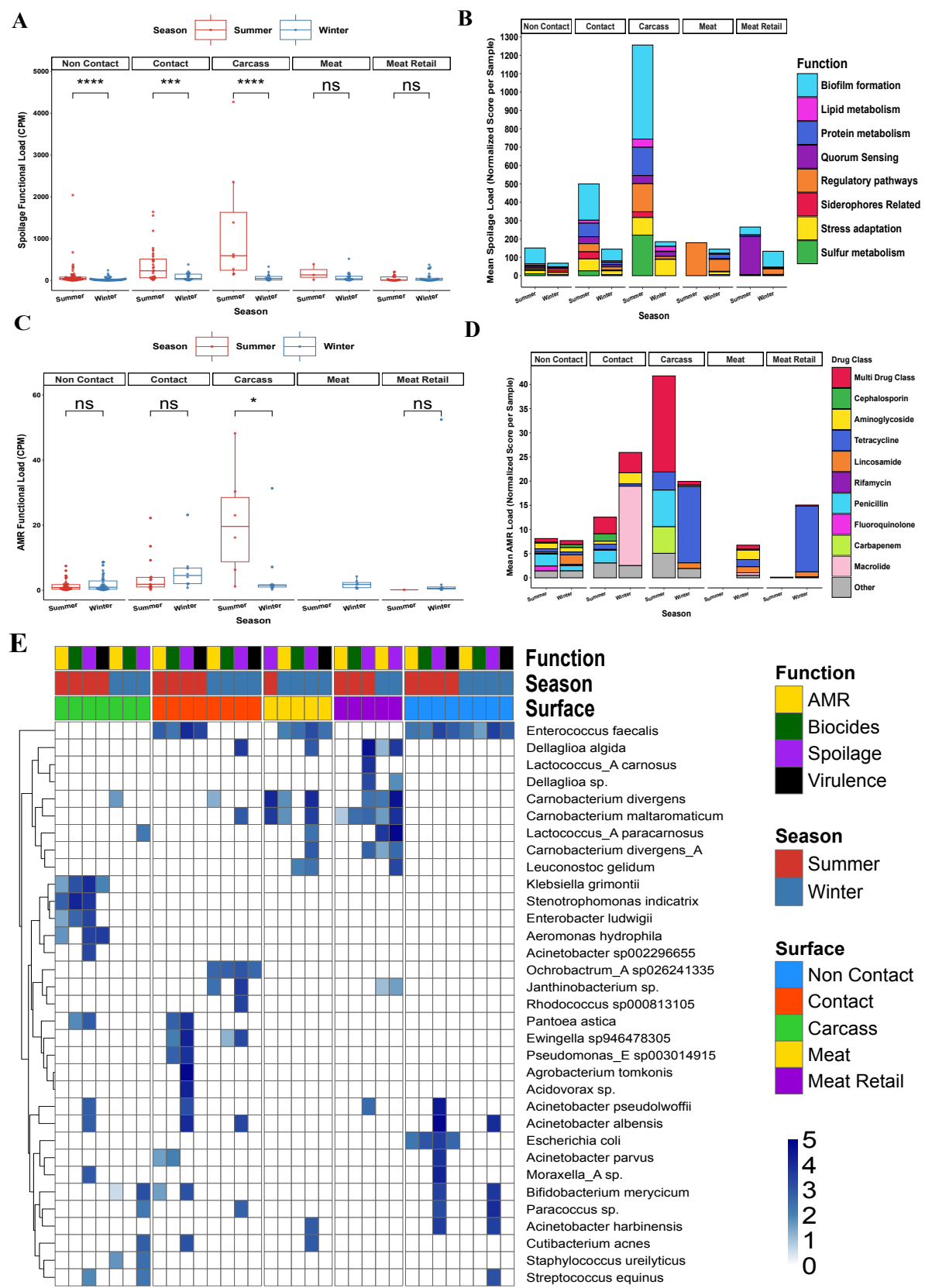


Figure 4: Abundance and composition of functional genes on processing surfaces and in final meat products across seasons. Gene load and composition for; (A, B) Spoilage genes

(C, D) Antimicrobial resistance genes. Boxplots were compared using the Wilcoxon Rank-Sum test. Asterisks indicate significance: $p < 0.05$ (*); $p < 0.01$ (**); $p < 0.001$ (***); $p < 0.0001$ (****). “ns” means not significant. (E) Heatmap of the top reservoir species carrying high functional gene loads across processing surfaces and final meat products. Gene loads are shown on a log₁₀ scale. The heatmap is filtered to display only the top 2 species from each niche (e.g., Carcass in Summer) that carry high functional loads and are shared across at least two niches.

3.4. Bacteriophages Act as a Key Ecological Driver for Structuring the Microbiome along the Production Chain

Alpha diversity analysis of phages showed a pronounced ecological gradient, similar to the previous results for bacterial alpha diversity across beef processing environments and matured meat products. Phages showed higher diversity at initial processing locations and on non-contact surfaces, which gradually decreased on contact and on carcasses in the later stages (Supplementary Figure 10A). In both maturations, richness gradually decreased over time across both seasons, while evenness gradually increased in both maturations from start to the end and showed higher evenness in winter as compared to summer (Supplementary Figure 10B).

Taxonomic profiling revealed that the phage community was dominated by phages targeting *E. coli*. Similarly, phages targeting *A. baumannii* were more prevalent and abundant in all processing locations and surfaces in summer. In addition, a stable core comprising phages targeting *B. subtilis*, *S. enterica*, *K. pneumoniae*, and *P. aeruginosa* was found to be prevalent and abundant at processing locations and surfaces (Supplementary Figure 10C & Figure 5A). Conversely, phages targeting *A. hydrophila*, *Brochothrix thermosphacta*, *C. divergens*, *L. lactis*, and *S. aureus* were more prevalent and abundant in both matured meat products across the seasons. Furthermore, phages targeting *E. coli* and *Lactococcus lactis* were found to be widespread across multiple meat maturation timepoints (Supplementary Figure 10C & Figure 5A).

Moreover, linear-mixed effects models were applied to correlate phage abundance against their associated bacterial host abundance. This revealed a strong positive correlation between phage and host abundance across all samples ($\beta = 0.48$, $p < 0.001$), independent of location and host identity. This relationship was even stronger in high-biomass samples (Figure 5B).

Likewise, using the same model across 818 different phage-host pairs at niche-specific levels again revealed significant positive associations. However, at this level, some phages showed a considerable suppression (phage predation) ($FDR < 0.05$) for particular hosts, including environmentalists and pathogens, both in processing locations and surfaces (Figure 5C & Supplementary Figure 10D). For instance, *L. monocytogenes* has been effectively suppressed ($\beta = -0.40$) on non-contact surfaces in the Slaughter Hall (Figure 5C & Supplementary Figure 10D). On matured meat products, this suppression was even more intensified: In continuous maturation, *L. monocytogenes* was significantly suppressed at Day 10 ($\beta = -0.73$), *S. aureus* at day 77 ($\beta = -0.39$), while *Levilactobacillus brevis* was suppressed at day 22 and 49 respectively (Figure 5D). In retail maturation, phages targeted spoilage-associated LAB such as *L. brevis* ($\beta = -2.61$), *L. paracasei* ($\beta = -0.98$); opportunistic pathogens such as *P. aeruginosa* ($\beta = -0.97$), *A. baumannii* ($\beta = -1.12$); and pathogens like *S. enterica* ($\beta = -0.62$) (Figure 5D).

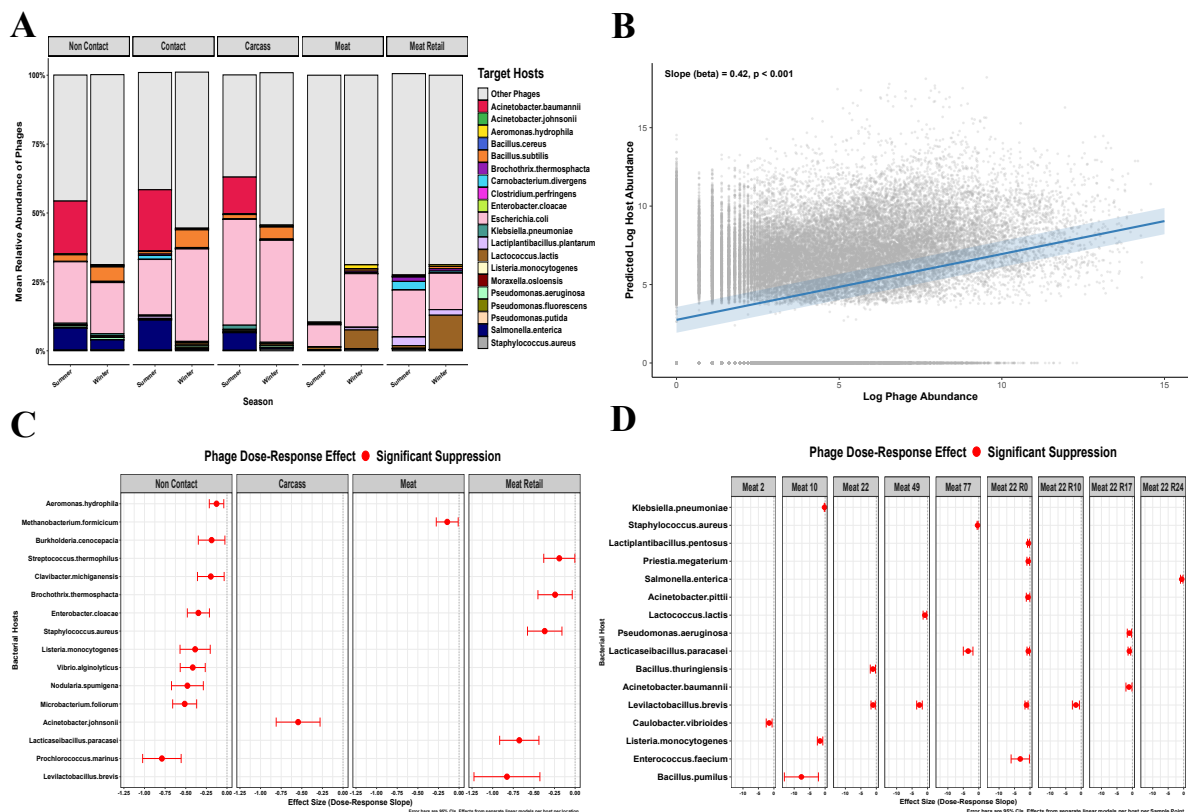


Figure 5: Abundance, composition, and activity of bacteriophages across processing surfaces and in final meat products across seasons. (A) Mean relative abundance (%) and composition of bacteriophages (targeted hosts names are shown in the legend) on processing surfaces and in meat products. **(B)** Linear-Mixed Effects model showing the association between host abundance and phage abundance across the whole data. **(C)** Suppression activity

of phages against their respective hosts on processing surfaces and final meat products. (D) Phage suppression activity against their respective hosts at each maturation time point.

3.5. Metagenome-Assembled Genomes (MAGs) Recovery and their Taxonomic Composition Across Processing Environments and Final Meat products

Overall, 4,475 medium-quality MAGs were recovered and retained for downstream analyses. Among these, 3,153 met high-quality criteria (Completeness ≥ 70 & Contamination ≤ 10) and 1,079 were classified as near-complete MAGs (Completeness ≥ 90 & Contamination ≤ 5) according to CheckM2. Each MAG represents a unique genome bin recovered from an individual sample. Detailed information for all medium-quality MAGs is provided in the supplementary material (Supplementary Table 3; Supplementary Figure 11). Interestingly, most genomes (MAGs = 2709; 60.5%) were identified as putative novel species not currently represented in the GTDB, while the remaining 1,766 (39.5%) were successfully assigned to known species. Notably, the genus *Corynebacterium* was the largest reservoir comprising 178 MAGs not assigned to any species by GTDB, followed by *Jeotgalicoccus* (n = 70), *Paracoccus* (n = 67). In addition to this high novelty, the overall taxonomic assignment revealed considerable diversity varying slightly between the seasons. A total of 320 unique genera and 340 distinct species were identified in summer, compared to 347 and 344 unique genera and species in winter.

Overall genus abundance also showed seasonal shifts. *Corynebacterium* was the most abundant genus found in both summer (n = 161) and winter (n = 182). However, other dominant genera varied across seasons, such as *Acinetobacter* (n = 68), *Latilactobacillus* (n = 53), and *Psychrobacter* (n = 52) were more prevalent in Summer. While *Bifidobacterium* (n = 104), *Latilactobacillus* (n = 98), and *Kocuria* (n = 80) were more prominent in winter.

Furthermore, the distribution of dominant genera across surfaces showed slight seasonal variations. For instance, during summer, *Corynebacterium* dominated both non-contact (n = 137) and contact (n = 18) surfaces, *Moraxella_A* on carcass (n = 8), while *Lactococcus* and *Latilactobacillus* dominated meat (n = 9) and meat retail (n = 41) products. In winter, *Corynebacterium* dominated non-contact (n = 125), Contact (n = 17), and carcass (n = 39) surfaces, while *Latilactobacillus* was again more prevalent on both meat (n = 32) and meat retail (n = 49) products.

Similarly, across locations in summer the dominant genera were *Rhodococcus* in (Farm = 5), *Corynebacterium* (Lairage Hall = 16, Slaughter Hall = 124, Boning Hall = 15), *Acinetobacter* (Chiller Hall = 11), and *Lactococcus* (Retail Hall = 11). While in winter, again *Corynebacterium* dominated all processing locations (Farm = 12, Lairage Hall = 14, Slaughter Hall = 99, Chiller Hall = 16, Boning Hall = 39), with *Latilactobacillus* dominating only the Retail Hall (n=16).

The species level seasonal differences across surfaces were also apparent. In summer, most abundant species recovered as MAGs on non-contact surfaces were *Acinetobacter pseudolwoffii* (n = 20), *Parageobacillus thermoglucosidasius_A* on contact (n = 17), *Klebsiella grimontii* on carcass (n = 3), *Carnobacterium maltaromaticum* and *Dellagليا algida* on meat (n = 6), and meat retail (n = 18) products. While in winter, these shifted to *Jeotgalicoccus sp003513765* (non-contact = 37), *Bifidobacterium globosum* (carcass = 20), and *Latilactobacillus fuchuensis* on (contact = 7, meat = 15, meat retail = 24).

Location-wise the dominant species in summer were, *Rhodococcus sp001426185* (Farm = 3), *Acinetobacter pseudolwoffii* (Lairage Hall = 6), *Bifidobacterium globosum* (Slaughter Hall = 18), *Acinetobacter harbinensis* (Chiller Hall = 5), *Parageobacillus thermoglucosidasius_A* (Boning Hall = 11), and *Lactococcus_A carnosus* in (Retail Hall = 11). During winter, the dominant species were shifted to *Bifidobacterium globosum* (Farm = 5, Slaughter Hall = 41, Chiller Hall = 8), *Jeotgalibaca porci* (Lairage Hall = 5), *Galactobacter caseinivorans* (Boning Hall = 14), and *Latilactobacillus fuchuensis* in (Retail Hall = 7). Figure 6 shows a detailed visual distribution of the most abundant species recovered as MAGs across the processing environment and final products in both seasons.

The sanitation impact was also analysed using recovered MAGs on species diversity and composition across seasons. In summer, the number of unique species increased substantially in post-cleaning (n = 118), compared to during production (n = 86). While in winter an opposite trend was observed, with species diversity decreasing from 168 unique species during production to 135 post-cleaning. Compositionally, the summer during production harboured *Microbacterium maritypicum* (n = 7), and *Acinetobacter harbinensis* (n = 6). After cleaning these were replaced by *Parageobacillus thermoglucosidasius_A* (14), *Agrobacterium tomkonis* (10), while *Microbacterium maritypicum* survived and increased from 7 to 10. In winter during production the dominant species were *Jeotgalicoccus sp003513765* (n = 11), and *Bifidobacterium globosum* (n = 10), which were then replaced by *Rothia sp002418375* (n =

10), *Acinetobacter albensis* (n = 8), and *Galactobacter caseinivorans* (n = 8) in post-cleaning (Supplementary Figure 12).

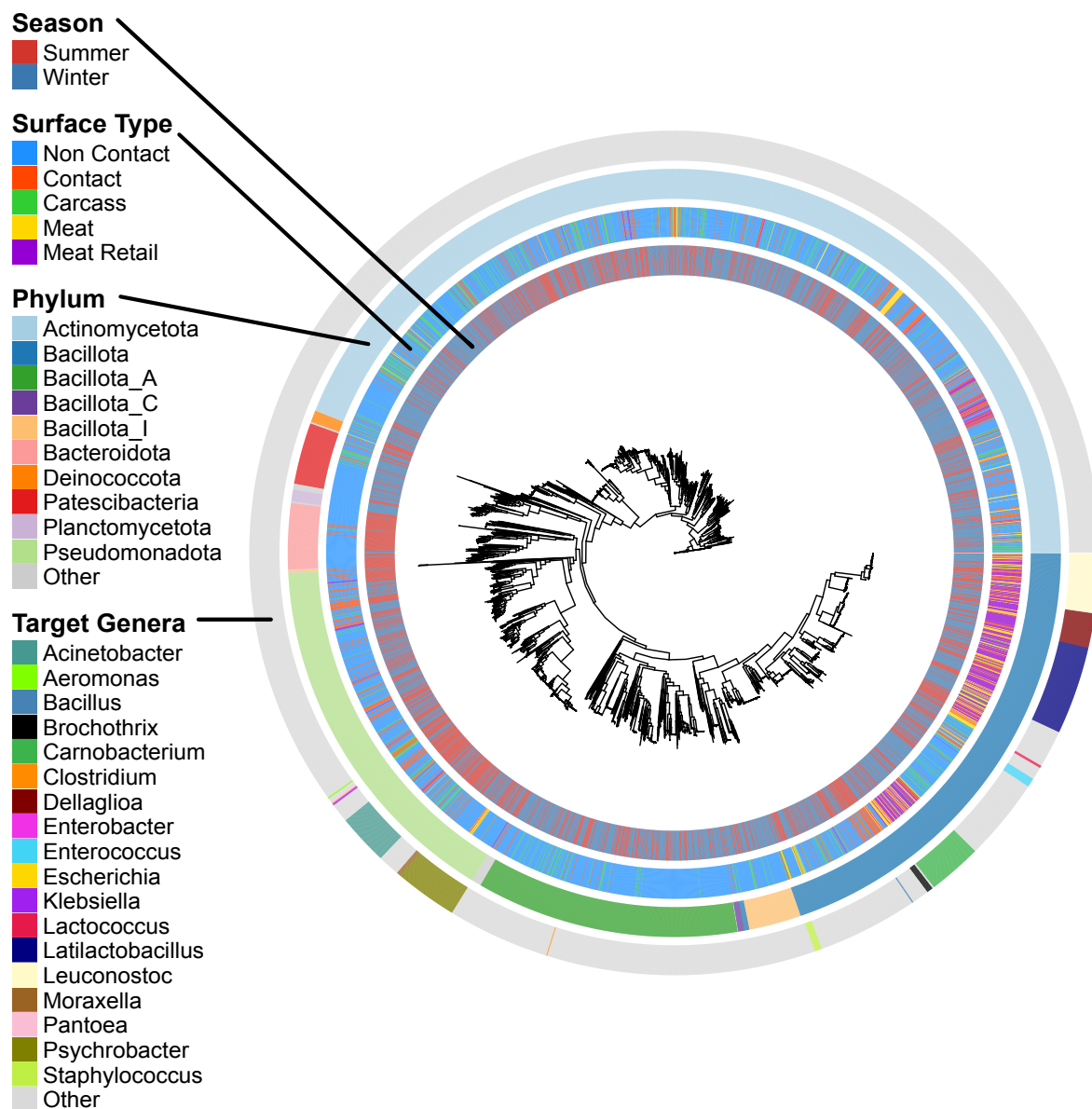


Figure 6: Phylogenomic tree of recovered MAGs showing their composition and distribution across seasons, processing surfaces and final meat products. The tree displays taxonomic composition of MAGs at phylum level; low abundance phyla are grouped as “Other” for visual clarity. The genera annotation highlights specific Target Genera (29 genera for meat-relevant spoilage and pathogenic species we followed in the taxonomic analysis at read-level). Vibrant colors show the 18 target genera recovered as MAGs. While the “Other” category groups all remaining genera, which includes both the 11 target genera not recovered, as well as all non-target genera.

4. Discussion:

The processing environment serves as a vast, dynamic reservoir that provides the primary microbial source of microbial contamination for the final products (Fagerlund et al., 2017; Zwirzitz et al., 2020; Barcenilla et al., 2024). The beta diversity analysis in this study showed well-separated cluster of environmental samples and meat products across seasons. Consistently with previous reports (Barcenilla et al., 2024; Palanisamy et al., 2023; Sequino et al., 2024), we observed a higher microbial diversity on surfaces than in meat samples.

In particular, the highest species richness was observed on surfaces during active production in the Slaughter Hall. However, this was not surprising, as slaughtering is a stage widely recognized as the most significant risk of cross-contamination (Nørrung et al., 2009; Wheatley et al., 2014) due to the contamination of the environment with microbiomes from mud, faeces, and accidental spillage during evisceration (Nørrung et al., 2009; Wheatley et al., 2014). On the contrary, the environmental samples from the Chiller Hall – where samples are stored at 0–3°C for 24 h – highlighted a low biodiversity, probably through selection of psychrotrophic and psychrotolerant species, as previously highlighted (Filippis et al., 2013).

Importantly, our data suggest that, beside food contact surfaces, operators and their knives might be relevant vectors for microbial transfer across the processing chains. Indeed, the prominent abundance of spoilage and pathogenic taxa (such as *S. enterica*) on hands and knives in the Boning Hall indicates that a cross-contamination event from the operator to the food might occur, shedding a light on the critical importance of hygiene control and operators' education. On the other side, the hygiene control of materials used for vacuum-packing the meat during maturation is crucial, as unsterile vacuum bags harboured a diverse microbial community that may act as vectors for recontamination, as also previously reported (Zwirzitz et al., 2021).

Throughout the process, the food microbiome faces dynamic changes. Indeed, the initial meat products harbour diverse microbial communities, likely reflecting early exposure to the processing environment, but maturation imposed a strong ecological filter, transforming a taxonomically diverse environmental microbiome into a highly specialized, low-diversity consortium, dominated by LAB. Our results were consistent with previous studies showing that vacuum packaging and prolonged low-temperature storage drive community toward a psychrotrophic LAB-dominated consortium (Freitas et al., 2024; Pennacchia et al., 2011; Roch

et al., 2025). This shift is particularly characterized by the enrichment of *C. divergens* and *C. maltaromaticum*, two species that are frequently abundant in long-term vacuum-packed meats across seasons (Leisner et al., 2007; Roch et al., 2025; Zhang et al., 2018) and exhibit well-documented proteolytic potential (Dallagnol et al., 2021; Quijada et al., 2022). Even though *Carnobacterium* spp. might protect the food from contamination by pathogens through bacteriocins production, as previously reported against many pathogenic species, including *L. monocytogenes* (Martin-Visscher et al., 2011; Zhang et al., 2019; Evangelista et al., 2023; Andrade Cavalari et al., 2024). Indeed, we observed enrichment for regulatory and protein metabolism pathways in meat during maturation, primarily driven by *Carnobacterium* species, demonstrating individualistic strategies necessary for survival in a harsh, low-nutrient environment (Roch et al., 2025; Sequino et al., 2024). Interestingly, retail storage induced a new change in meat microbiome composition, where *Carnobacterium* spp. were displaced by *L. carnosus* and *L. paracarnosus*, potentially reflecting changes associated with mincing or portioning procedures during the retail processing (Cauchie et al., 2020; Roch et al., 2025).

Interestingly, high-risk pathogens such as *S. enterica*, *L. monocytogenes*, and *S. aureus* were consistently detected in meat products, but at very low mean relative abundances (<0.2%) across maturation processes and seasons. This might be linked to bioprotective effects exerted by the LAB (Dallagnol et al., 2021; Fernandes et al., 2024; Gontijo et al., 2022; Koutsoumanis et al., 2024), to the high prevalence of bacteriophages targeting the pathogens, or to a combination of these factors.

Furthermore, the processing environment revealed a clear seasonal partition in species richness. Our data showed higher species richness in summer, which likely reflects animal management practices such as open-field rearing, where cattle are exposed to a broader environmental microbiome, particularly on animal hides. This diverse microbial input subsequently seeds the processing environment. In contrast, the reduced richness observed during winter is consistent with indoor housing practices, where animals are kept in close contact, promoting homogenization of gut and skin microbiota and limiting exposure to external environmental sources. Additionally, the warmer and more humid summer conditions within the processing environment may further enhance microbial persistence and activity. Ultimately, this results in a more versatile taxonomic structure and functional gene potential. Accordingly, the summer Chiller Hall and associated carcasses displayed the highest loads of functional risks, including spoilage, biocide resistance, and virulence potential. These findings align with observations

from poultry processing systems, where temperature-driven seasonal variation strongly shaped functional gene profiles (Michelle Baker et al., 2023).

One of the most critical findings of this study is that sanitation failed to substantially alter the core microbiome across processing stages and seasons, jeopardizing the cleaning and decontamination efforts. Rather than simply resetting the core community, it exerted additional selective pressure, reshaping the house microbiome in paradoxical ways. These findings align with previous reports, demonstrating the persistence of microbial consortia on processing surfaces that resist standard cleaning regimes (Ban-Cucerzan et al., 2025; Chowdhury et al., 2025; Palanisamy et al., 2023).

However, sanitation showed targeted efficacy; for instance, in a retail hall, it suppressed some LAB species and *Clostridium perfringens*, an anaerobe that can thrive in vacuum-packed meat products. However, this success was contrasted by post-cleaning enrichment of some opportunistic biofilm-forming taxa, such as *A. baumannii* and *E. faecium*, reflecting the phenomenon of competitive release, in which the removal of susceptible competitors creates open slots for the enrichment of resilient taxa (Sui et al., 2023; Wang et al., 2024).

Functionally, this post-cleaning also showed a dramatic increase in biocides, AMR, virulence, and spoilage genes in several locations and surfaces, particularly a >3.2-fold increase was observed for biofilm forming genes, consistent with the previous findings, that post-cleaning surfaces pose a high risk for biofilm forming taxa carrying AMR and virulence genes (Fagerlund et al., 2021; Zwirzitz et al., 2021, 2020).

Seasonal effects also amplified this functional risk, for instance, in summer, post-cleaning surfaces showed the highest abundance of genes linked to spoilage (regulatory pathways), biocide resistance (Benzalkonium Chloride, Hydrogen Peroxide), virulence (immune modulation, adherence), and multidrug class AMR. In some cases, total microbial load decreased, but disproportionality increased in high-risk functional traits. This dual and paradoxical impact is well-supported by previous studies (Cadena et al., 2019). Collectively, these findings emphasize that cleaning is not a neutral event; rather, it is an ecological perturbation that reshapes microbial communities and their functions. Understanding this dynamic is crucial for designing interventions that do not merely reduce the total load but also target the resilient mechanisms that allow biofilm-forming, high-risk organisms to thrive.

Bacteriophages are well-known for their ecological role in reshaping and managing bacterial diversity in complex ecosystems, and that's why we're interested in uncovering this unexplored potential in the beef processing environment and in matured meat products. Our results showed a striking paradoxical impact of bacteriophage dynamics along the whole processing chain. Across the entire dataset, phage abundance showed a strong positive correlation with that of their bacterial hosts. This aligns with the well-known Piggyback-the-Winner (PtW) hypothesis, which posits that phages maintain stable coexistence with their bacterial hosts under certain ecological conditions (Silveira and Rohwer, 2016; Voigt et al., 2021). However, in meat processing environments, such positive relationships illustrate the role of biofilm-rich house microbiome, where biofilms restrict phage diffusions, also the replacement of susceptible host strains by the resistant ones of the same species (Pires et al., 2021), resulting in the simultaneous survival of predator and prey, triggering an apparent system-wide positive signal. Moreover, acknowledging the inherent limitations of computational phage predictions and strain-level resolution of our data might be another possible explanation for this positive signal.

However, in-depth characterization of these phage-host relationships revealed interesting, but localized, strong negative associations between predator and prey, consistent with the Kill-the-Winner (KtW) hypothesis. Where phages kill their abundant hosts, a model proposed by Thingstad (2000) and extended by Silveira & Rohwer (2016) (Silveira and Rohwer, 2016; Thingstad, 2000). Notably, these localized interactions were equally spatially structured across processing environments and matured meat products. For instance, phages targeting pathogens such as *L. monocytogenes* were suppressed in both the processing environment and meat products. While *S. aureus* and *S. enterica* were suppressed exclusively on meat products, suggesting their potential role as natural biocontrol agents, a finding consistent with prior observations in food environments (de Melo et al., 2018; Moye et al., 2018). These findings also support the view that phage-host dynamics in food systems are profoundly context-dependent, modulated by microenvironmental structure, temporal dynamics, and host resistance traits (Kavagutti et al., 2019; Liu et al., 2025).

Finally, this ecological duality, from system-wide coexistence to localized suppression, illustrates that phages are not merely passive bystanders but active agents shaping microbial structuring in beef processing environments. However, the triggers that determine whether a phage remains temperate or becomes lytic are most likely context-dependent, influenced by factors such as host density, resilience, biofilm refuges, sanitizer exposure, nutrient availability,

and environmental stress (Fortier and Sekulovic, 2013). Similarly, these environmental triggers of phage switching from the piggybacking model to active killers are still widely disputed (Hu et al., 2025). Conclusively, the observed positive correlations and localized suppressive effects reflect a balance between coexistence, resistance evolution, and periodic host turnover. From an applied perspective, these findings have important implications for phage-based biocontrol strategies because of their power, sustainability, and eco-friendly approach to disinfecting the processing environment without harming the environment.

Metagenome-assembled genomes (MAGs) analysis provided a genome-resolved layer of evidence that reinforced our core ecological patterns detected from read-level and contig-based profiling. MAGs recovery was markedly greater in processing environment ($n = 4029$) than in the final meat products across seasons. Likewise, the non-contact surfaces in Slaughter Hall had the highest number of recovered MAGs, classified as unique species (summer = 225 vs. winter = 192), supporting the idea that the processing environments, particularly in summer sustained more diverse and functionally flexible communities. Similar observations have been widely reported in meat processing chains, where processing surfaces act as microbial reservoirs (Barcenilla et al., 2024; Sequino et al., 2025). Importantly, the environmental taxa we identified as key functional reservoirs, including *Klebsiella grimontii*, *Aeromonas hydrophila*, *Enterococcus faecalis*, *Stenotrophomonas indicatrix*, and *Acinetobacter albensis* were also recovered as MAGs across seasons, validating that these organisms were not sequencing artefacts but active community members with high metabolic potential, especially during the summer period.

In contrast, the matured meat products yielded far fewer MAGs ($n = 446$) across seasons, in which 374 MAGs were assigned to LAB genera, including *Carnobacterium*, *Lactococcus*, and *Latilactobacillus*, *Dellagloba*, and *Leuconostoc*, consistent with our read-level observations and also by others (Barcenilla et al., 2024). However, no MAGs were recovered corresponding to the high-priority pathogens, including *Clostridium perfringens*, *Klebsiella pneumoniae*, *Listeria monocytogenes*, *Staphylococcus aureus*, *Salmonella enterica*, *Campylobacter jejuni*, *Campylobacter coli*, and *Clostridium botulinum* in matured meat products. Thus, the lack of MAG recovery and extremely low read abundance in meat products, strongly implies that the persistent low-level signals we observed previously in our read-level analysis, represent non-viable “relic DNA” from the upstream processing line rather than an active proliferating population, a pattern consistent with previous studies (Carini et al., 2016).

Overall, the strong concordance between the read-level taxonomic profiling, contig-level functional signatures, and the MAG-level data resolution strengthens our findings, that prolonged wet-age maturation imposes strong selective pressures, thereby reducing the biohazards while enriching the spoilage-associated LAB communities. This multi-layered confirmation elevates our confidence in the ecological framework proposed for meat-chain microbiomes.

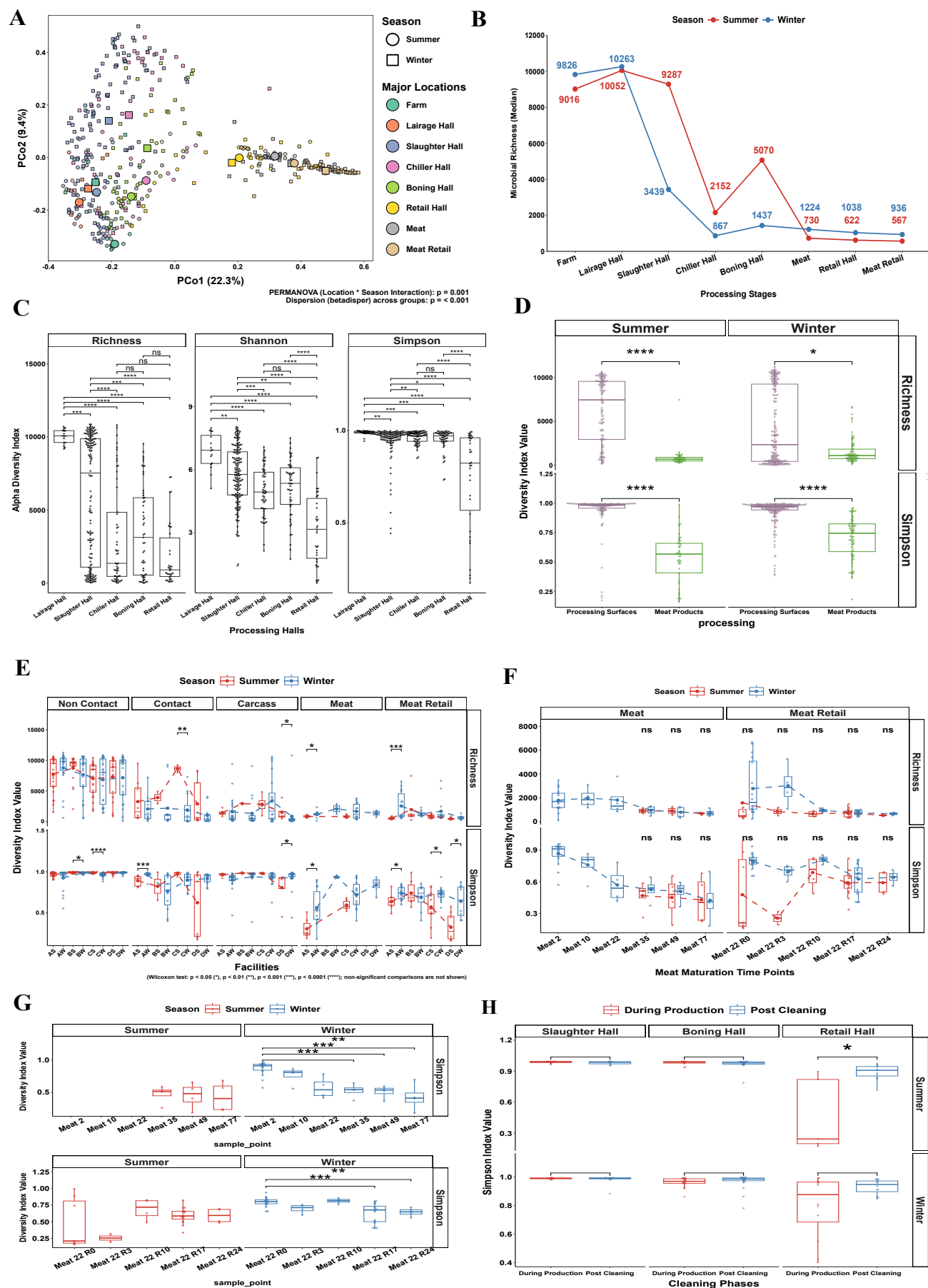
Conclusion

This longitudinal farm-to-fork investigation reframes the beef processing chain as a living ecosystem rather than a sterile and sedentary industrial pipeline. By tracing the same animals along the entire processing chains across two seasons, this study provides the first integrative evidence that seasonality, sanitation, and phage activity collectively shape the stepwise progression of beef microbiomes through competition, selective pressures, and viral predation. Seasonal contrasts were striking: the summer environment supported higher microbial and functional diversity within the processing environment, whereas winter favoured more diverse communities on matured meat products. These shifts underscore that microbial risks in meat production are dynamic, climate-sensitive, and shaped by initial load shaped by local herd management practices. Despite an overall stable community structure on matured meat products, different maturation protocols produced distinct microbial outcomes across seasons. Notably, wet aging under vacuum at sub-zero temperatures enhances product stability by minimizing myoglobin oxidation (Imazaki et al., 2023), improving tenderness and flavour (Yu et al., 2024), and reducing microbial hazards (Koutsoumanis et al., 2024). In this study, it was also associated with a negligible functional risk profile and active phage suppression of key meat pathogens, ultimately highlighting its positive impact on meat quality, safety, and prolonged shelf life.

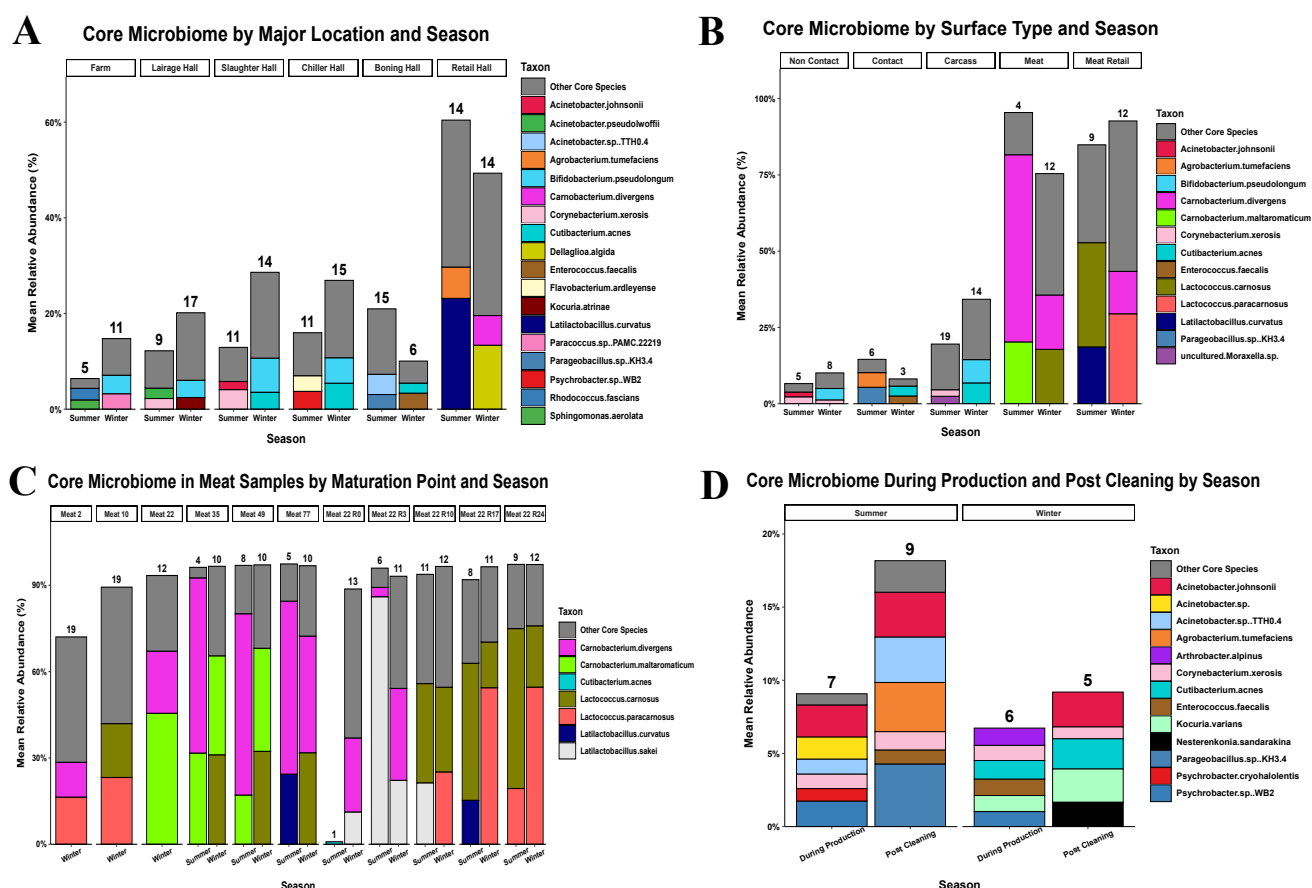
Limitations and Future Perspectives:

Although this study offers an unprecedented high-resolution view of microbial succession along the beef processing continuum across contrasting seasons, several constraints should be recognized. First, by design, the metagenomics reveals the genetic potential rather than the active metabolic activity or physiological state of microbial communities. Subsequently, the functional and phage-host associations identified here illuminate the computational predictions that remain to be experimentally validated using culture-based or transcriptomic approaches. Similarly, the facilities studied here operate in temperate climates and high-throughput beef production systems typical of Northern Europe, which may not fully capture microbial dynamics in other regions or management contexts. Likewise, each facility we investigated came from a single farm batch, limiting the representativeness of upstream microbial variability across farms. However, despite these constraints, this work establishes a comprehensive ecological framework for understanding the impacts of seasons, sanitation, and bacteriophage activity on microbial succession and functional filtering in the modern meat processing chains.

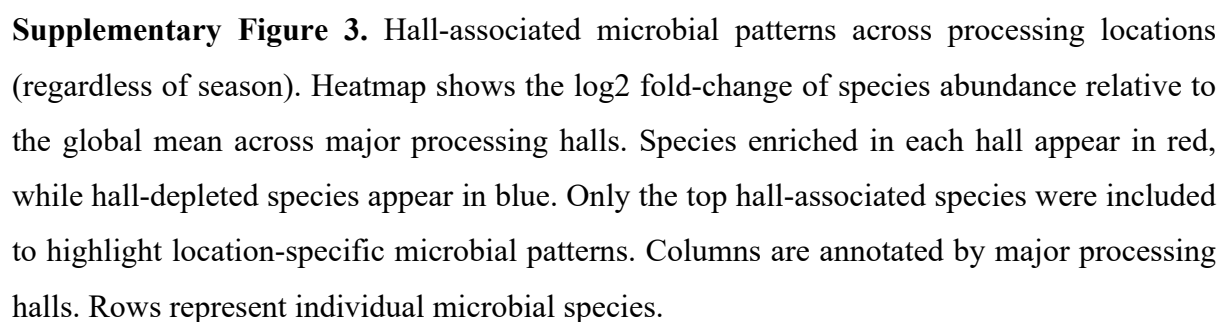
The next frontier is to move beyond genomic potential towards functional validation. Integrating meta-transcriptomics and metabolomics will directly link microbial presence with metabolic activity, connecting gene expression and metabolite production to sensory quality, spoilage dynamics, and safety outcomes. Likewise, coupling these approaches with advanced culturomics will enable isolation and experimental validation of phage-host interactions deduced here, clarifying their ecological roles in microbiome management. Furthermore, translating these insights into targeted applications, such as developing multi-phage cocktails to suppress key meat-relevant pathogens selectively, offers a natural alternative to conventional chemical sanitizers, reducing the environmental dissemination of AMR in industrial effluents. Moreover, the introduction of complementary interventions, such as plasma-activated water or antimicrobial packaging, could further enhance product quality, safety, and shelf life. Ultimately, this work paves the way for a paradigm shift in meat science: from the pursuit of sterility to the design of a safer, more sustainable, and ecologically balanced food system.

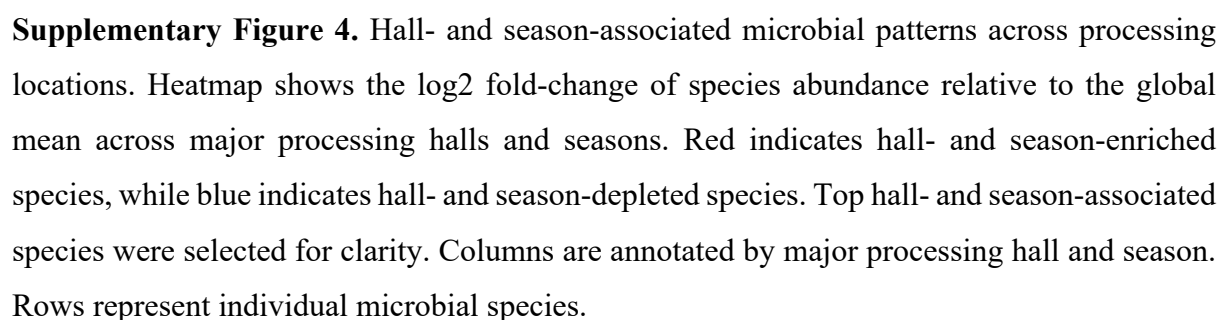


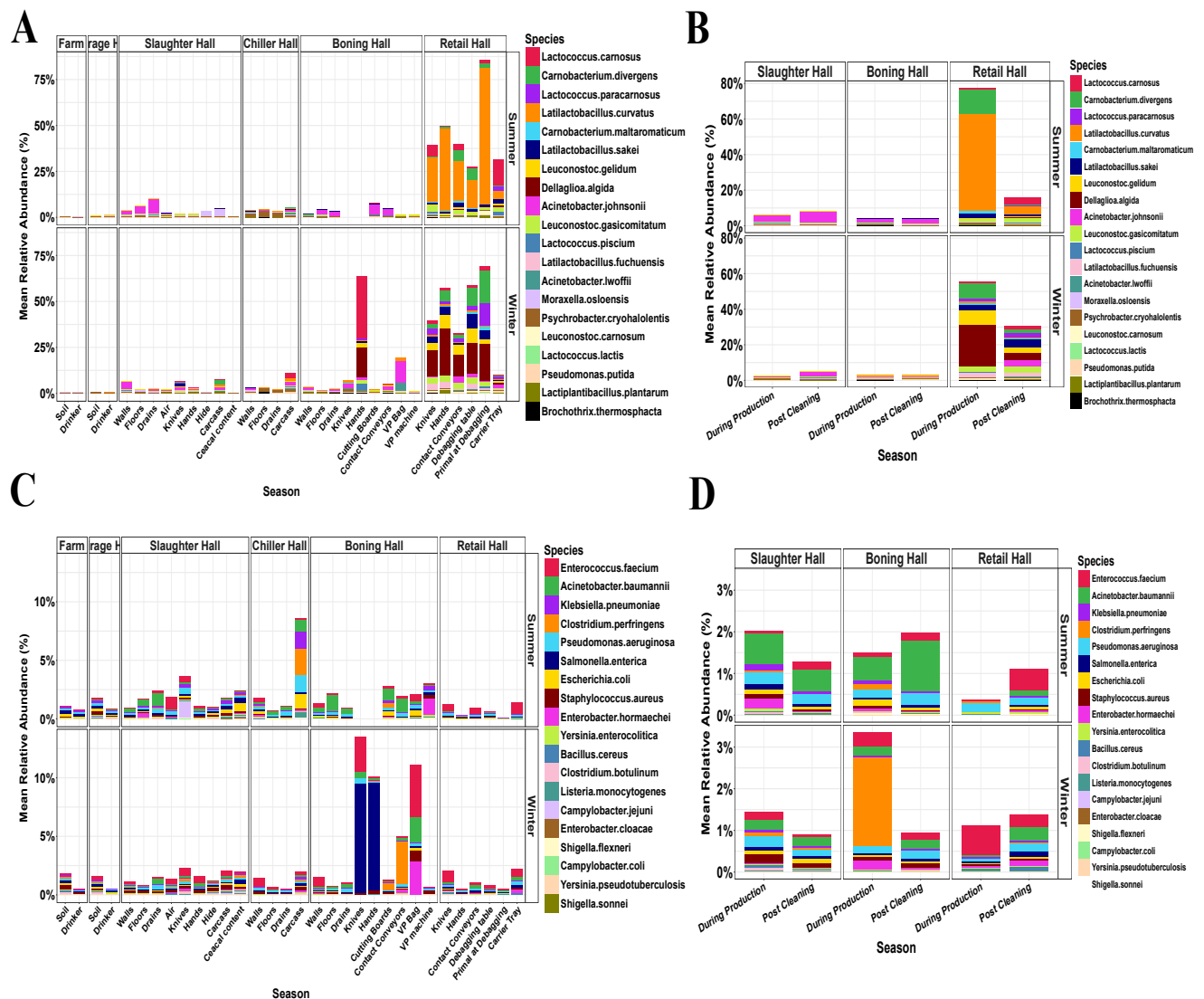
Supplementary Figure 1: Alpha and Beta Diversity Analysis: (A) PCoA ordination (using Bray-Curtis dissimilarity) comparing microbial communities across processing locations, where colours represent locations and shapes represent seasons. (B) A gradual decrease in observed richness (Median value) across processing locations and Seasons. (C) Alpha diversity indices of all processing locations (processing halls) regardless of season. (D) Alpha diversity (Richness & Simpson Index) of all processing surfaces against all meat products within each seasons separately. (E) Alpha diversity (Richness & Simpson Index) comparing processing surfaces and final meat products across different facilities and seasons. (F) Seasonal comparisons of alpha diversity (Richness and Simpson Index) for final products (Meat and Meat Retail) at each timepoint. (G) Simpson Index for both meat products, showing pairwise comparisons between the initial timepoint and later timepoints, analysed within each season separately. (H) Alpha diversity (Simpson Index) comparing samples from “During Production” versus “Post cleaning” for each processing location and season. Boxplots were compared using the Wilcoxon Rank-Sum test with Benjamini-Hochberg correction. Asterisks indicate significance: $p < 0.05$ (*); $p < 0.01$ (**); $p < 0.001$ (***); $p < 0.0001$ (****). “ns” means not significant (most non-significant comparisons are not shown).



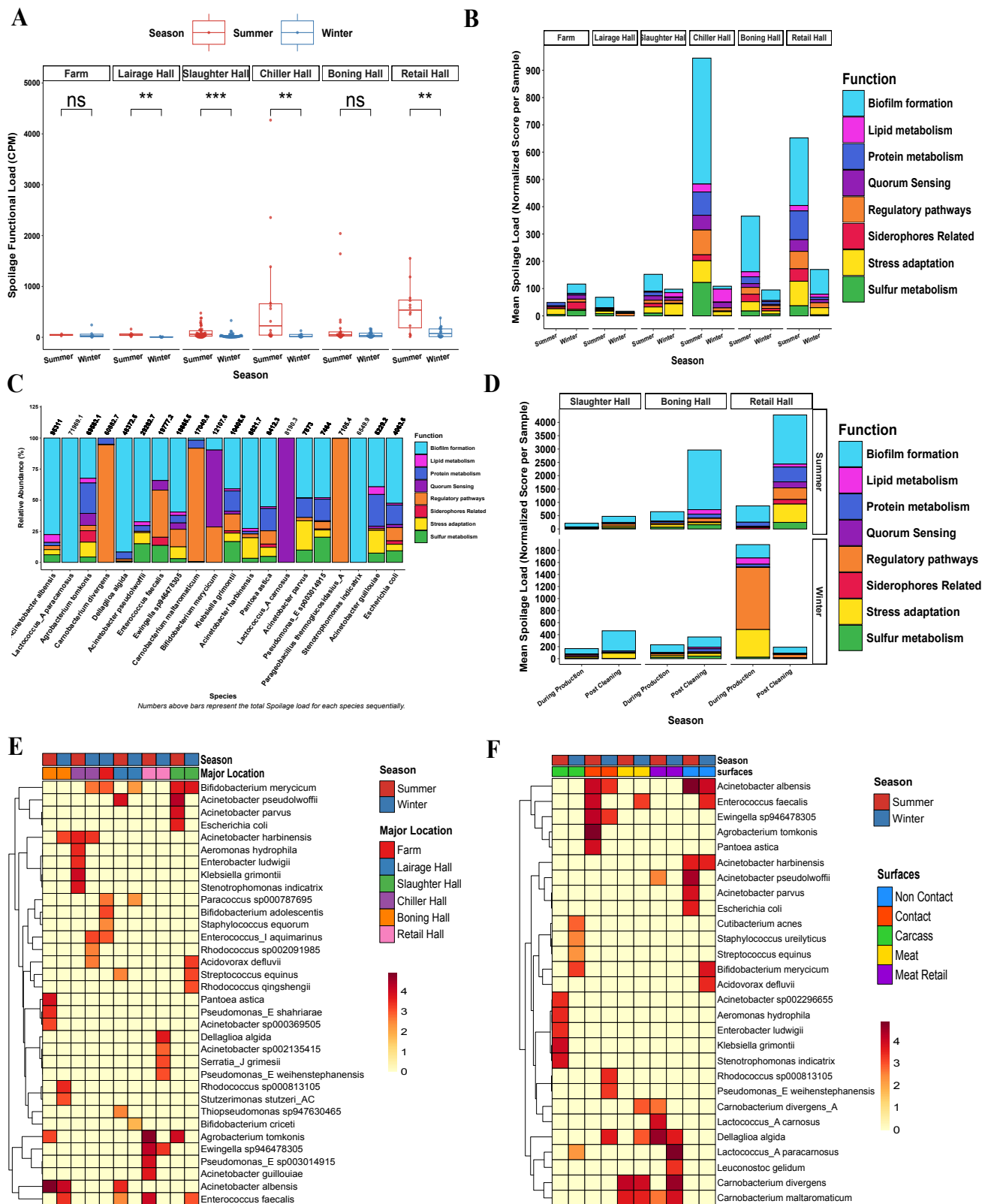
Supplementary Figure 2: Taxonomic Composition and Mean Relative Abundance of the Core Microbiome. This figure displays the core microbiome, with the top two most abundant species in each niche highlighted in colour and all other core species shown in grey. The number above each stacked bar indicates the total count of core microbial species identified for that specific niche. (A) Mean relative abundance (%) of core microbial species, comparing seasons across processing locations. (B) Mean relative abundance (%) of core microbial species, comparing seasons across processing surfaces and final meat products. (C) Mean relative abundance (%) of core microbial species, comparing each maturation timepoint for both Meat and Meat Retail products across seasons. (D) Mean relative abundance (%) of core microbial species, comparing “During Production” versus “Post-Cleaning” samples within each season.





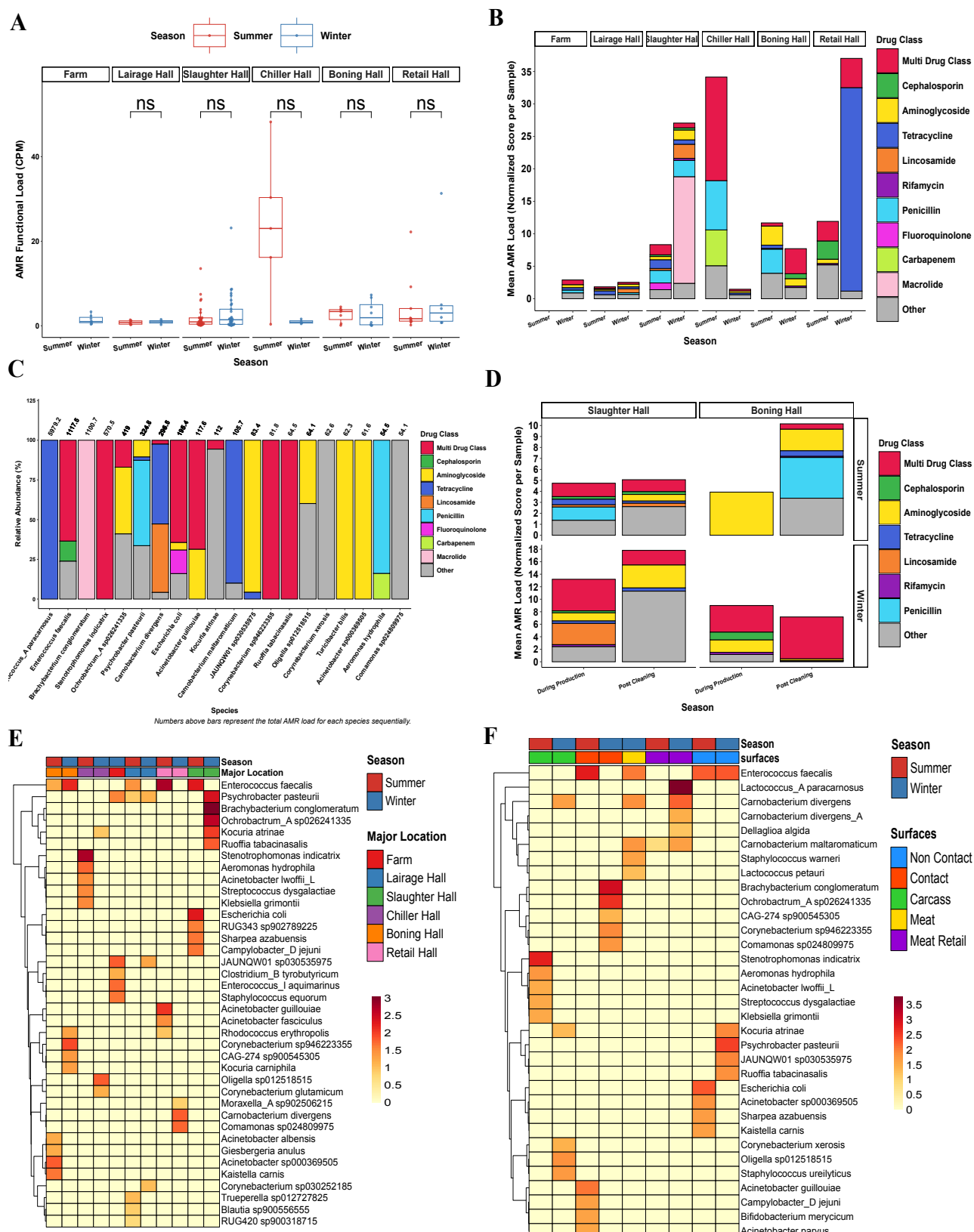


Supplementary Figure 5: Taxonomic Composition and Mean Relative Abundance of Meat-Relevant Spoilers and Pathogens. (A) Mean relative abundance (%) of spoilers at each sample point across processing locations and seasons. (B) Mean relative abundance (%) of spoilers, comparing “During Production” versus “Post-Cleaning” samples within each processing location and season. (C) Mean relative abundance (%) of pathogens at each sample point across processing locations and seasons. (D) Mean relative abundance (%) of pathogens, comparing “During Production” versus “Post-Cleaning” samples within each processing location and season.



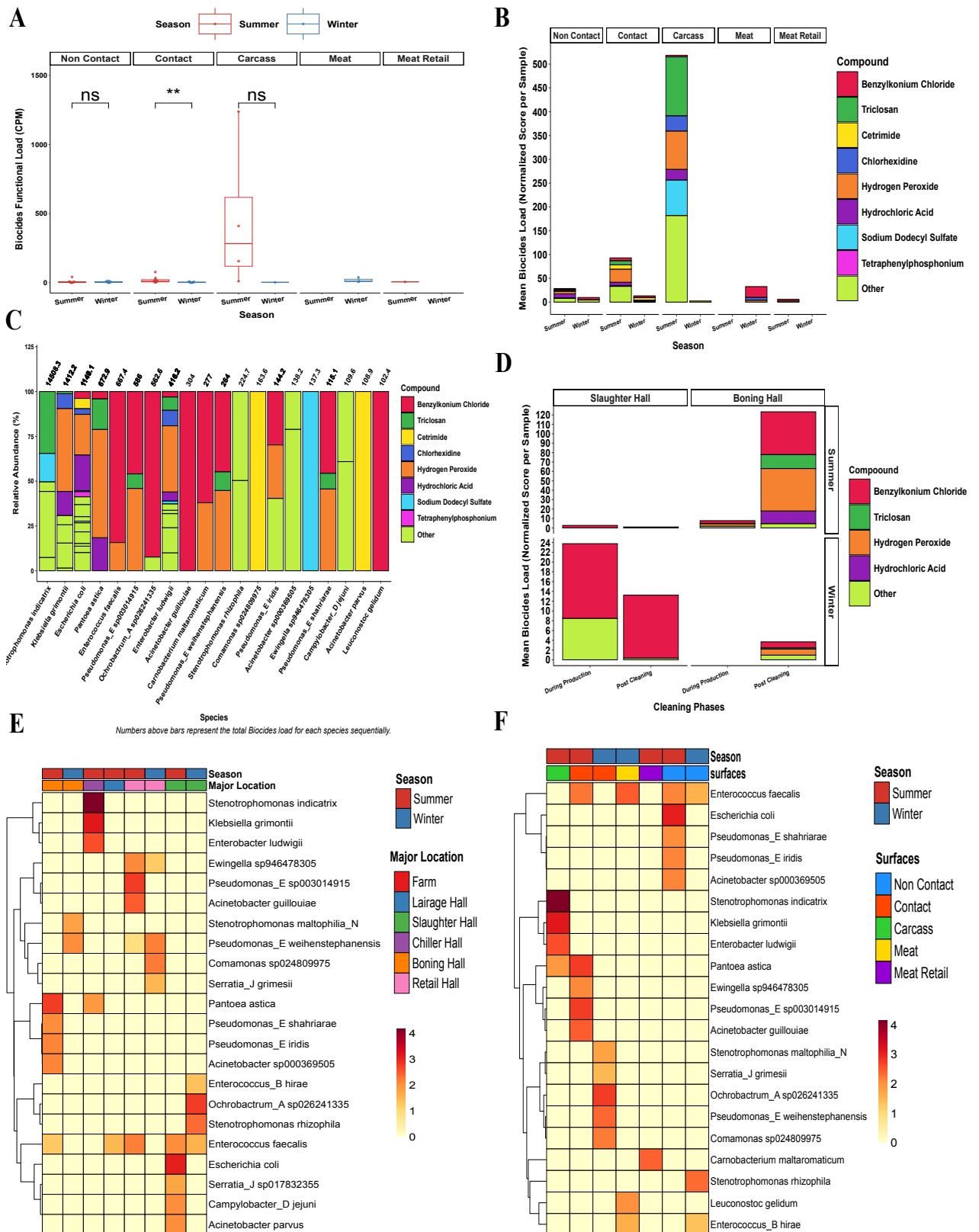
Supplementary Figure 6: Abundance and Composition of Spoilage-Related Genes. (A) Spoilage functional load, comparing seasons across processing locations. (B) Composition and mean normalized load of spoilage genes, comparing seasons at each individual maturation

timepoint in both Meat and Meat Retail. (C) Stacked bar plot of the top 20 reservoir species, ranked by their total quantified spoilage gene load. Each bar is segmented to show the composition of spoilage gene categories within that species. The total spoilage load (score) for each species is noted above the bar, and species are sorted from highest (left) to lowest (right). For instance, *Lactococcus paracarnosus* having the second highest average spoilage gene load all 100% corresponding to biofilm formation genes detected/located in Meat Retail products (D) Composition and mean normalized load of spoilage genes, comparing “During Production” vs. “Post-Cleaning” at each processing location, analyzed separately by season. (E) Heatmap showing the distribution and detection of top reservoir species carrying significant spoilage gene loads across processing locations and seasons. (F) Heatmap showing the distribution and detection of top reservoir species carrying significant spoilage gene loads across processing surfaces and seasons. For all panels, boxplots or bars are not shown for groups or timepoints where no spoilage genes were detected. Boxplots were compared using the Wilcoxon Rank-Sum test with Benjamini-Hochberg correction. Asterisks indicate significance: $p < 0.05$ (*), $p < 0.01$ (**), $p < 0.001$ (***), $p < 0.0001$ (****). “ns” indicates non-significance (most non-significant comparisons are not shown).



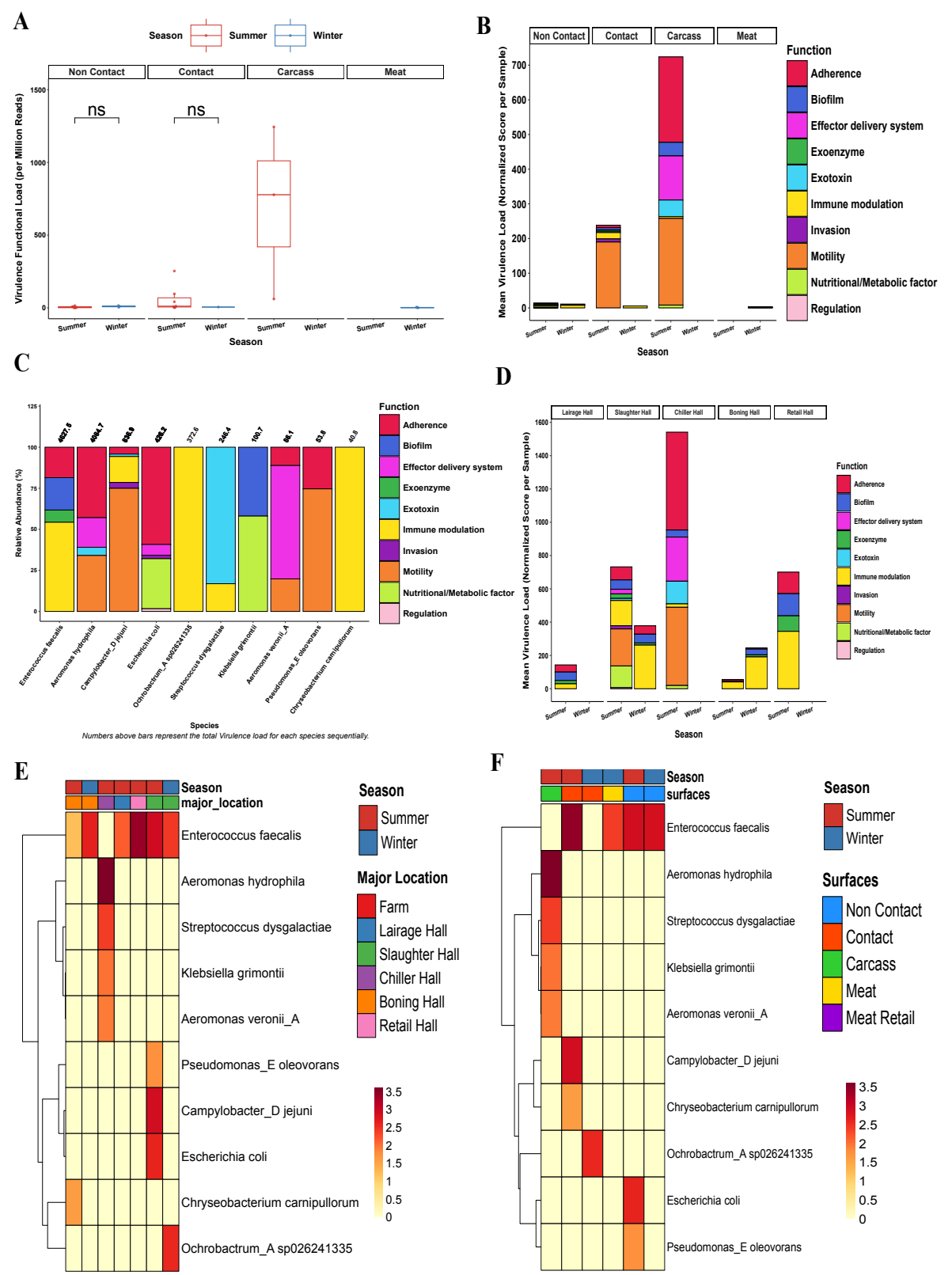
Supplementary Figure 7: Abundance and Composition of Antimicrobial Resistance (AMR) Genes. (A) Abundance of AMR functional load, comparing seasons across processing locations. (B) Composition and mean normalized load of AMR genes, comparing seasons

across processing locations. (C) Stacked bar plot of the top 20 reservoir species, ranked by their total quantified AMR gene load. Each bar is segmented to show the gene composition of that load within each species. The total AMR load (score) for each species is noted above the bar, and species are sorted from highest (left) to lowest (right). For instance *Enterococcus faecalis* was found to be the second highest species carrying a significant AMR load composed of nearly all drug classes which was detected across multiple locations and surfaces. (D) Composition and mean normalized load of AMR genes, comparing “During Production” vs. “Post-Cleaning” at each processing location, analysed separately by season. (E) Heatmap showing the distribution and detection of top reservoir species carrying significant AMR gene loads across processing locations and seasons. (F) Heatmap showing the distribution and detection of top reservoir species carrying significant AMR gene loads across processing surfaces and seasons. For all panels, boxplots or bars are not shown for groups or timepoints where no AMR genes were detected. Boxplots were compared using the Wilcoxon Rank-Sum test with Benjamini-Hochberg correction. Asterisks indicate significance: $p < 0.05$ (*), $p < 0.01$ (**), $p < 0.001$ (***), $p < 0.0001$ (****). “ns” indicates non-significance (most non-significant comparisons are not shown).



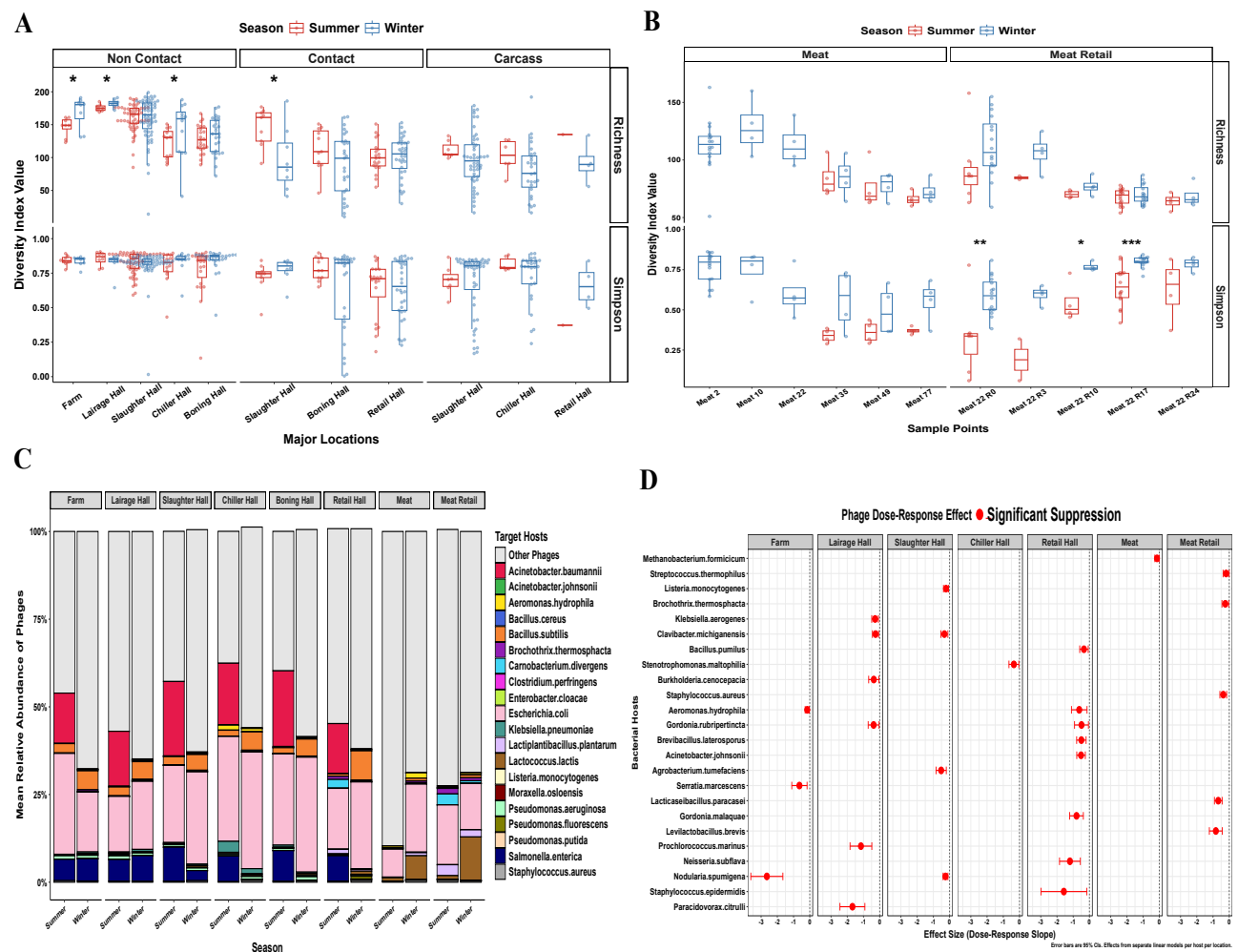
Supplementary Figure 8: Abundance and Composition of Biocide Genes. (A) Abundance of biocide functional load, comparing seasons across processing surfaces and meat products. (B) Composition and mean normalized load of biocide genes (common sanitizers and

disinfectants), comparing seasons across processing surfaces and meat products. (C) Stacked bar plot of the top 20 reservoir species, ranked by their total quantified biocide gene load. Each bar is segmented to show the gene composition of that load within each species. The total biocide load (score) for each species is noted above the bar, and species are sorted from highest (left) to lowest (right). (D) Composition and mean normalized load of biocide genes, comparing “During Production” vs. “Post-Cleaning” at each processing location, analysed separately by season. (E) Heatmap showing the distribution and detection of top reservoir species carrying significant biocide gene loads across processing locations and seasons. (F) Heatmap showing the distribution and detection of top reservoir species carrying significant biocide gene loads across processing surfaces and seasons. For all panels, boxplots or bars are not shown for groups or timepoints where no biocide genes were detected. Boxplots were compared using the Wilcoxon Rank-Sum test with Benjamini-Hochberg correction. Asterisks indicate significance: $p < 0.05$ (*), $p < 0.01$ (**), $p < 0.001$ (***), $p < 0.0001$ (****). “ns” indicates non-significance (most non-significant comparisons are not shown).

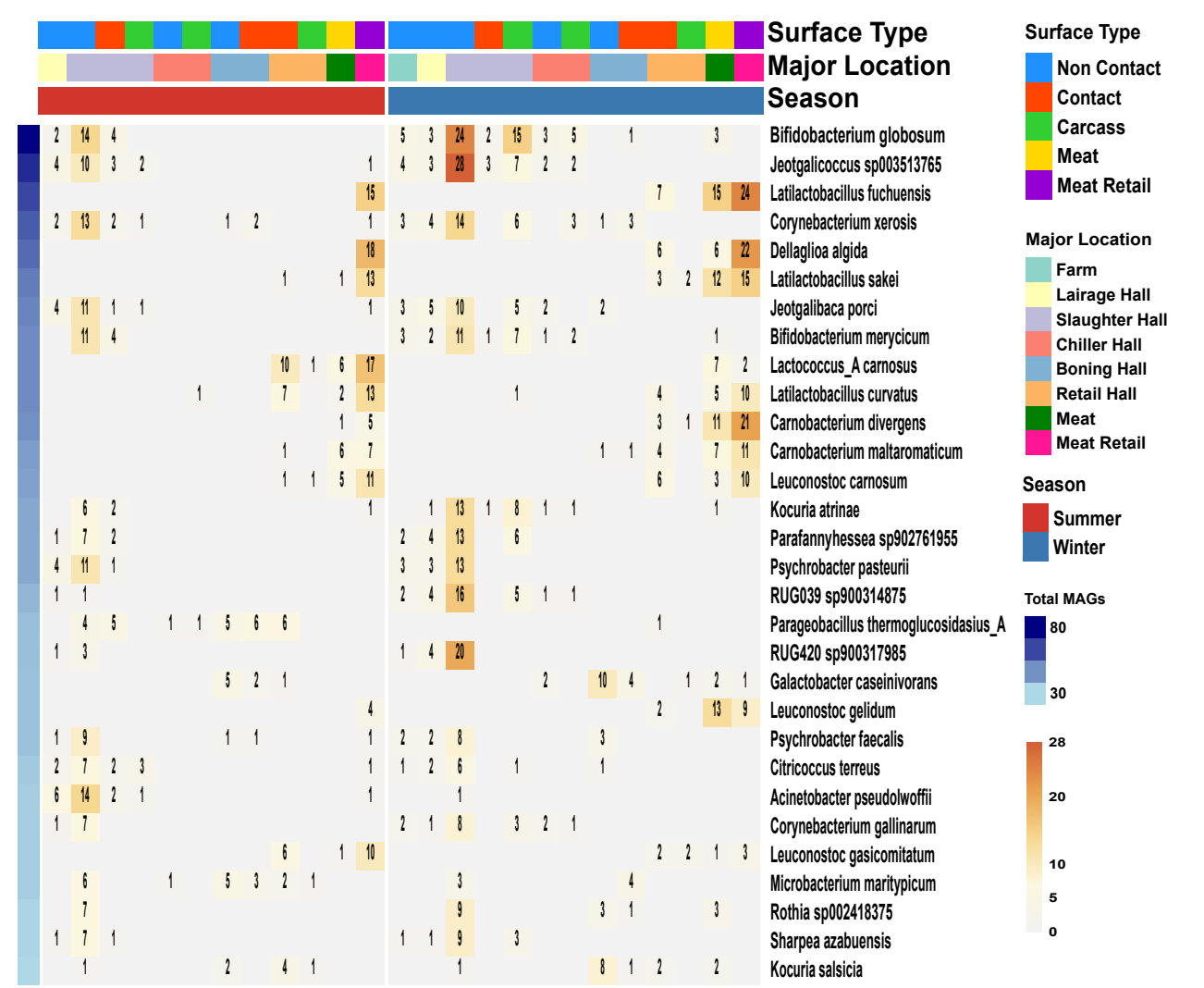


Supplementary Figure 9: Abundance and Composition of Virulence Genes. (A) Abundance of virulence functional load, comparing seasons across processing surfaces and meat products. (B) Composition and mean normalized load of virulence genes, comparing seasons across

processing surfaces and meat products. (C) Stacked bar plot of the top 20 reservoir species, ranked by their total quantified virulence gene load. Each bar is segmented to show the gene composition of that load within each species. The total virulence load (score) for each species is noted above the bar, and species are sorted from highest (left) to lowest (right). (D) Composition and mean normalized load of virulence genes, comparing “During Production” vs. “Post-Cleaning” at each processing location, analyzed separately by season. (E) Heatmap showing the distribution and detection of top reservoir species carrying significant virulence gene loads across processing locations and seasons. (F) Heatmap showing the distribution and detection of top reservoir species carrying significant virulence gene loads across processing surfaces and seasons. For all panels, boxplots or bars are not shown for groups or timepoints where no virulence genes were detected. Boxplots were compared using the Wilcoxon Rank-Sum test with Benjamini-Hochberg correction. Asterisks indicate significance: $p < 0.05$ (*), $p < 0.01$ (**), $p < 0.001$ (***), $p < 0.0001$ (****). “ns” indicates non-significance (most non-significant comparisons are not shown).



Supplementary Figure 10: Bacteriophage Alpha Diversity, Composition, and Host Suppression. (A) Alpha diversity (Richness and Simpson Index) on surfaces at each processing location. (B) Alpha diversity (Richness and Simpson Index) of phage communities, comparing seasons at each individual meat maturation timepoint. (C) Taxonomic composition and mean relative abundance (%) of phages, grouped by their predicted bacterial hosts, across seasons, processing locations, and final meat products. (D) Significant suppressive interactions (predation analysis) between bacteriophages and their respective hosts at specific processing locations and on final meat products.



Supplementary Figure 11: Heatmap showing the distribution of top 30 most abundant species recovered as MAGs across processing surfaces, major locations, final meat products, and seasons. The MAGs are assigned at the species level. The numbers in each cell represent the total count of recovered MAGs in that specific niche (e.g., a non-contact surface in the slaughter hall during summer).



Supplementary Figure 12: Heatmap of the top 30 recovered MAG species, comparing “During Production” vs. “Post-Cleaning” samples within each season.

References:

- Alcock, B.P., Huynh, W., Chalil, R., Smith, K.W., Raphenya, A.R., Wlodarski, M.A., Edalatmand, A., Petkau, A., Syed, S.A., Tsang, K.K., Baker, S.J.C., Dave, M., McCarthy, M.C., Mukiri, K.M., Nasir, J.A., Golbon, B., Imtiaz, H., Jiang, X., Kaur, K., Kwong, M., Liang, Z.C., Niu, K.C., Shan, P., Yang, J.Y.J., Gray, K.L., Hoad, G.R., Jia, B., Bhando, T., Carfrae, L.A., Farha, M.A., French, S., Gordzevich, R., Rachwalski, K., Tu, M.M., Bordeleau, E., Dooley, D., Griffiths, E., Zubyk, H.L., Brown, E.D., Maguire, F., Beiko, R.G., Hsiao, W.W.L., Brinkman, F.S.L., Van Domselaar, G., McArthur, A.G., 2023. CARD 2023: expanded curation, support for machine learning, and resistome prediction at the Comprehensive Antibiotic Resistance Database. *Nucleic Acids Res* 51, D690–D699. <https://doi.org/10.1093/nar/gkac920>
- Ban-Cucerzan, A., Imre, K., Morar, A., Marcu, A., Hotea, I., Popa, S.-A., Pătrînjă, R.-T., Bucur, I.-M., Gașpar, C., Plotuna, A.-M., Ban, S.-C., 2025. Persistent Threats: A Comprehensive Review of Biofilm Formation, Control, and Economic Implications in Food Processing Environments. *Microorganisms* 13, 1805. <https://doi.org/10.3390/microorganisms13081805>
- Barcenilla, C., Cobo-Díaz, J.F., Puente, A., Valentino, V., De Filippis, F., Ercolini, D., Carlino, N., Pinto, F., Segata, N., Prieto, M., López, M., Alvarez-Ordóñez, A., 2024. In-depth characterization of food and environmental microbiomes across different meat processing plants. *Microbiome* 12, 199. <https://doi.org/10.1186/s40168-024-01856-3>
- BenLangmead, n.d. Kraken 2, KrakenUniq and Bracken indexes. URL <https://benlangmead.github.io/aws-indexes/k2> (accessed 3.15.25).
- Benoit, G., Raguideau, S., James, R., Phillippy, A.M., Chikhi, R., Quince, C., 2024. High-quality metagenome assembly from long accurate reads with metaMDBG. *Nat Biotechnol* 42, 1378–1383. <https://doi.org/10.1038/s41587-023-01983-6>
- Borges, F., Briandet, R., Callon, C., Champomier-Vergès, M.-C., Christeans, S., Chuzeville, S., Denis, C., Desmaures, N., Desmonts, M.-H., Feurer, C., Leroi, F., Leroy, S., Mounier, J., Passerini, D., Pilet, M.-F., Schlusshuber, M., Stahl, V., Strub, C., Talon, R., Zagorec, M., 2022. Contribution of omics to biopreservation: Toward food microbiome engineering. *Front Microbiol* 13, 951182. <https://doi.org/10.3389/fmicb.2022.951182>
- Cadena, M., Kelman, T., Marco, M.L., Pitesky, M., 2019. Understanding Antimicrobial Resistance (AMR) Profiles of Salmonella Biofilm and Planktonic Bacteria Challenged

- with Disinfectants Commonly Used During Poultry Processing. *Foods* 8, 275. <https://doi.org/10.3390/foods8070275>
- Carini, P., Marsden, P.J., Leff, J.W., Morgan, E.E., Strickland, M.S., Fierer, N., 2016. Relic DNA is abundant in soil and obscures estimates of soil microbial diversity. *Nat Microbiol* 2, 16242. <https://doi.org/10.1038/nmicrobiol.2016.242>
- Carneiro, K.O., Campos, G.Z., Scafuro Lima, J.M., Rocha, R. da S., Vaz-Velho, M., Todorov, S.D., 2024. The Role of Lactic Acid Bacteria in Meat Products, Not Just as Starter Cultures. *Foods* 13, 3170. <https://doi.org/10.3390/foods13193170>
- Casaburi, A., Piombino, P., Nychas, G.-J., Villani, F., Ercolini, D., 2015. Bacterial populations and the volatilome associated to meat spoilage. *Food Microbiology, Spoilers, wonder spores and diehard microorganisms: New insights to integrate these super foes in food spoilage risk management* 45, 83–102. <https://doi.org/10.1016/j.fm.2014.02.002>
- Cauchie, E., Delhalle, L., Taminiau, B., Tahiri, A., Korsak, N., Berteau, S., Fall, P.A., Farnir, F., Baré, G., Daube, G., 2020. Assessment of Spoilage Bacterial Communities in Food Wrap and Modified Atmospheres-Packed Minced Pork Meat Samples by 16S rDNA Metagenetic Analysis. *Front Microbiol* 10, 3074. <https://doi.org/10.3389/fmicb.2019.03074>
- Chaumeil, P.-A., Mussig, A.J., Hugenholtz, P., Parks, D.H., 2020. GTDB-Tk: a toolkit to classify genomes with the Genome Taxonomy Database. *Bioinformatics* 36, 1925–1927. <https://doi.org/10.1093/bioinformatics/btz848>
- Chklovski, A., Parks, D.H., Woodcroft, B.J., Tyson, G.W., 2023. CheckM2: a rapid, scalable and accurate tool for assessing microbial genome quality using machine learning. *Nat Methods* 20, 1203–1212. <https://doi.org/10.1038/s41592-023-01940-w>
- Chowdhury, M.A.H., Reem, C.S.A., Ashrafudoulla, Md., Rahman, Md.A., Shaila, S., Jie-won Ha, A., Ha, S.-D., 2025. Role of advanced cleaning and sanitation techniques in biofilm prevention on dairy equipment. *Comprehensive Reviews in Food Science and Food Safety* 24, e70176. <https://doi.org/10.1111/1541-4337.70176>
- Cobo-Díaz, J.F., Alvarez-Molina, A., Alexa, E.A., Walsh, C.J., Mencía-Ares, O., Puente-Gómez, P., Likotrafiti, E., Fernández-Gómez, P., Prieto, B., Crispie, F., Ruiz, L., González-Raurich, M., López, M., Prieto, M., Cotter, P., Alvarez-Ordóñez, A., 2021. Microbial colonization and resistome dynamics in food processing environments of a newly opened pork cutting industry during 1.5 years of activity. *Microbiome* 9, 204. <https://doi.org/10.1186/s40168-021-01131-9>

- Dallagnol, A.M., Pescuma, M., Espínola, N.G., Vera, M., Vignolo, G.M., 2021. Hydrolysis of raw fish proteins extracts by *Carnobacterium maltaromaticum* strains isolated from Argentinean freshwater fish. *Biotechnology Reports* 29, e00589. <https://doi.org/10.1016/j.btre.2021.e00589>
- de Andrade Cavalari, C.M., Imazaki, P.H., Pirard, B., Lebrun, S., Vanleyssem, R., Gemmi, C., Antoine, C., Crevecoeur, S., Daube, G., Clinquart, A., de Macedo, R.E.F., 2024. *Carnobacterium maltaromaticum* as bioprotective culture against spoilage bacteria in ground meat and cooked ham. *Meat Science* 211, 109441. <https://doi.org/10.1016/j.meatsci.2024.109441>
- De Filippis, F., Valentino, V., Alvarez-Ordóñez, A., Cotter, P.D., Ercolini, D., 2021. Environmental microbiome mapping as a strategy to improve quality and safety in the food industry. *Current Opinion in Food Science* 38, 168–176. <https://doi.org/10.1016/j.cofs.2020.11.012>
- De Filippis, F., Valentino, V., Yap, M., Cabrera-Rubio, R., Barcenilla, C., Carlino, N., Cobo-Díaz, J.F., Quijada, N.M., Calvete-Torre, I., Ruas-Madiedo, P., Sabater, C., Sequino, G., Pasolli, E., Wagner, M., Margolles, A., Segata, N., Álvarez-Ordóñez, A., Cotter, P.D., Ercolini, D., 2024. Microbiome mapping in dairy industry reveals new species and genes for probiotic and bioprotective activities. *npj Biofilms Microbiomes* 10, 67. <https://doi.org/10.1038/s41522-024-00541-5>
- de Melo, A.G., Levesque, S., Moineau, S., 2018. Phages as friends and enemies in food processing. *Current Opinion in Biotechnology, Food biotechnology • Plant biotechnology* 49, 185–190. <https://doi.org/10.1016/j.copbio.2017.09.004>
- Debray, R., Herbert, R.A., Jaffe, A.L., Crits-Christoph, A., Power, M.E., Koskella, B., 2022. Priority effects in microbiome assembly. *Nat Rev Microbiol* 20, 109–121. <https://doi.org/10.1038/s41579-021-00604-w>
- Fagerlund, A., Langsrud, S., Møretrø, T., 2021. Microbial diversity and ecology of biofilms in food industry environments associated with *Listeria monocytogenes* persistence. *Current Opinion in Food Science* 37, 171–178. <https://doi.org/10.1016/j.cofs.2020.10.015>
- FAO, 2013. Food wastage footprint: impacts on natural resources summary report, Organisation des Nations Unies pour l'alimentation et l'agriculture. ed. FAO, Rome.
- Fernandes, N., Achemchem, F., Gonzales-Barron, U., Cadavez, V., 2024. Biopreservation strategies using bacteriocins to control meat spoilage and foodborne outbreaks. *Ital J Food Saf* 13, 12558. <https://doi.org/10.4081/ijfs.2024.12558>

- Filippis, F.D., Storia, A.L., Villani, F., Ercolini, D., 2013. Exploring the Sources of Bacterial Spoilers in Beefsteaks by Culture-Independent High-Throughput Sequencing. *PLOS ONE* 8, e70222. <https://doi.org/10.1371/journal.pone.0070222>
- Fortier, L.-C., Sekulovic, O., 2013. Importance of prophages to evolution and virulence of bacterial pathogens. *Virulence* 4, 354–365. <https://doi.org/10.4161/viru.24498>
- Frank, D., Hughes, J., Piyasiri, U., Zhang, Y., Kaur, M., Li, Y., Mellor, G., Stark, J., 2020. Volatile and non-volatile metabolite changes in 140-day stored vacuum packaged chilled beef and potential shelf life markers. *Meat Science* 161, 108016. <https://doi.org/10.1016/j.meatsci.2019.108016>
- Freitas, C.F. de, Carvalho, L.M.V.F., Camargo, A.C., Almeida, O.G.G. de, Oliveira, R.R. de, De Martinis, E.P., Yamatogi, R.S., Nero, L.A., 2024. Bacterial microbiota shifts in vacuum-packed beef during storage at different temperatures: Impacts on blown pack spoilage. *Food Microbiology* 119, 104448. <https://doi.org/10.1016/j.fm.2023.104448>
- Fukami, T., Mordecai, E.A., Ostling, A., 2016. A framework for priority effects. *Journal of Vegetation Science* 27, 655–657. <https://doi.org/10.1111/jvs.12434>
- ggplot2, n.d. An implementation of the Grammar of Graphics in R. URL <https://github.com/tidyverse/ggplot2> (accessed 10.25.24).
- Gontijo, M.T.P., Ramia, N.E., Dijamentiuk, A., Elfassy, A., Taha, S., Mangavel, C., Revol-Junelles, A.-M., Borges, F., 2022. Mining Biosynthetic Gene Clusters in *Carnobacterium maltaromaticum* by Interference Competition Network and Genome Analysis. *Microorganisms* 10, 1794. <https://doi.org/10.3390/microorganisms10091794>
- Guron, G.K.P., Cassidy, J.M., Chen, C.-Y., Paoli, G.C., 2024. Transfer of beef bacterial communities onto food-contact surfaces. *Front Microbiol* 15, 1450682. <https://doi.org/10.3389/fmicb.2024.1450682>
- Hempel, C.A., Wright, N., Harvie, J., Hleap, J.S., Adamowicz, S.J., Steinke, D., 2022. Metagenomics versus total RNA sequencing: most accurate data-processing tools, microbial identification accuracy and perspectives for ecological assessments. *Nucleic Acids Res* 50, 9279–9293. <https://doi.org/10.1093/nar/gkac689>
- Hernandez, M.S., Woerner, D.R., Brooks, J.C., Wheeler, T.L., Legako, J.F., 2022. Influence of Aging Temperature and Duration on Spoilage Organism Growth, Proteolytic Activity, and Related Chemical Changes in Vacuum-Packaged Beef Longissimus. *Meat and Muscle Biology* 6. <https://doi.org/10.22175/mmb.13724>
- Hernández-Macedo, M.L., Contreras-Castillo, C.J., Tsai, S.M., Da Cruz, S.H., Sarantopoulos, C.I.G.L., Padula, M., Dias, C.T.S., 2012. Gases and volatile compounds associated with

- micro-organisms in blown pack spoilage of Brazilian vacuum-packed beef. *Lett. Appl. Microbiol.* 55, 467–475. <https://doi.org/10.1111/lam.12004>
- Hu, C., Chen, X., Wei, W., Wallace, D., Liu, J., Zhang, Y., Zhang, L., Xu, D., Batt, J., Xiao, X., Shi, Q., Zheng, Q., Ma, R., Luo, T., Jiao, N., Zhang, R., 2025. To kill or to piggyback: Switching of viral lysis-lysogeny strategies depending on host dynamics. *Science of The Total Environment* 959, 178233. <https://doi.org/10.1016/j.scitotenv.2024.178233>
- Imazaki, P.H., Taminiau, B., Fall, P.A., Elansary, M., Douny, C., Scippo, M.-L., Daube, G., Clinquart, A., 2023. Oxidative Stability and Microbial Ecology of Fresh Beef with Extremely Long Shelf-Life. *Journal of Food Processing and Preservation* 2023, 5319266. <https://doi.org/10.1155/2023/5319266>
- Kang, D.D., Li, F., Kirton, E., Thomas, A., Egan, R., An, H., Wang, Z., 2019. MetaBAT 2: an adaptive binning algorithm for robust and efficient genome reconstruction from metagenome assemblies. *PeerJ* 7, e7359. <https://doi.org/10.7717/peerj.7359>
- Karwowska, M., Łaba, S., Szczepański, K., 2021. Food Loss and Waste in Meat Sector—Why the Consumption Stage Generates the Most Losses? *Sustainability* 13, 6227. <https://doi.org/10.3390/su13116227>
- Kavagutti, V.S., Andrei, A.-Ş., Mehrshad, M., Salcher, M.M., Ghai, R., 2019. Phage-centric ecological interactions in aquatic ecosystems revealed through ultra-deep metagenomics. *Microbiome* 7, 135. <https://doi.org/10.1186/s40168-019-0752-0>
- Koutsoumanis, K., Allende, A., Bolton, D., Bover-Cid, S., Chemaly, M., De Cesare, A., Herman, L., Hilbert, F., Lindqvist, R., Nauta, M., Nonno, R., Peixe, L., Ru, G., Simmons, M., Skandamis, P., Suffredini, E., Fox, E., Gosling, R. (Becky), Gil, B.M., Møretrø, T., Stessl, B., da Silva Felício, M.T., Messens, W., Simon, A.C., Alvarez-Ordóñez, A., 2024. Persistence of microbiological hazards in food and feed production and processing environments. *EFSA J* 22, e8521. <https://doi.org/10.2903/j.efsa.2024.8521>
- Langmead, B., Salzberg, S.L., 2012. Fast gapped-read alignment with Bowtie 2. *Nat Methods* 9, 357–359. <https://doi.org/10.1038/nmeth.1923>
- Leisner, J.J., Laursen, B.G., Prévost, H., Drider, D., Dalgaard, P., 2007. *Carnobacterium*: positive and negative effects in the environment and in foods. *FEMS Microbiol Rev* 31, 592–613. <https://doi.org/10.1111/j.1574-6976.2007.00080.x>
- Lennon, J.T., Muscarella, M.E., Placella, S.A., Lehmkuhl, B.K., 2018. How, When, and Where Relic DNA Affects Microbial Diversity. *mBio* 9, e00637-18. <https://doi.org/10.1128/mBio.00637-18>

- Li, D., Liu, C.-M., Luo, R., Sadakane, K., Lam, T.-W., 2015. MEGAHIT: an ultra-fast single-node solution for large and complex metagenomics assembly via succinct de Bruijn graph. *Bioinformatics* 31, 1674–1676. <https://doi.org/10.1093/bioinformatics/btv033>
- Liu, B., Bai, M., Tu, W., Shen, Y., Liu, J., Yang, Z., Bao, H., Dong, Q., Liu, Y., Wang, R., Zhang, H., Hu, L., 2025. The Virulence Factor LLO of *Listeria monocytogenes* Can Hamper Biofilm Formation and Indirectly Suppress Phage-Lytic Effect. *Foods* 14, 2554. <https://doi.org/10.3390/foods14152554>
- Liu, B., Zheng, D., Zhou, S., Chen, L., Yang, J., 2022. VFDB 2022: a general classification scheme for bacterial virulence factors. *Nucleic Acids Res* 50, D912–D917. <https://doi.org/10.1093/nar/gkab1107>
- Lu, J., Breitwieser, F.P., Thielen, P., Salzberg, S.L., 2017. Bracken: estimating species abundance in metagenomics data. *PeerJ Computer Science* 3, e104. <https://doi.org/10.7717/peerj-cs.104>
- Moye, Z.D., Woolston, J., Sulakvelidze, A., 2018. Bacteriophage Applications for Food Production and Processing. *Viruses* 10, 205. <https://doi.org/10.3390/v10040205>
- Nørrung, B., Andersen, J.K., Buncic, S., 2009. Main Concerns of Pathogenic Microorganisms in Meat, in: Toldrá, F. (Ed.), *Safety of Meat and Processed Meat*. Springer, New York, NY, pp. 3–29. https://doi.org/10.1007/978-0-387-89026-5_1
- Ondov, B.D., Treangen, T.J., Melsted, P., Mallonee, A.B., Bergman, N.H., Koren, S., Phillippy, A.M., 2016. Mash: fast genome and metagenome distance estimation using MinHash. *Genome Biol* 17, 132. <https://doi.org/10.1186/s13059-016-0997-x>
- Pal, C., Bengtsson-Palme, J., Rensing, C., Kristiansson, E., Larsson, D.G.J., 2014. BacMet: antibacterial biocide and metal resistance genes database. *Nucleic Acids Res* 42, D737–D743. <https://doi.org/10.1093/nar/gkt1252>
- Palanisamy, V., Bosilevac, J.M., Barkhouse, D.A., Velez, S.E., Chitlapilly Dass, S., 2023. Shotgun-metagenomics reveals a highly diverse and communal microbial network present in the drains of three beef-processing plants. *Front. Cell. Infect. Microbiol.* 13. <https://doi.org/10.3389/fcimb.2023.1240138>
- Park, J. min, Koh, J.H., Cho, M.J., Kim, J.M., 2020. Transfer rates of pathogenic bacteria during pork processing. *J Anim Sci Technol* 62, 912–921. <https://doi.org/10.5187/jast.2020.62.6.912>
- Pasolli, E., Asnicar, F., Manara, S., Zolfo, M., Karcher, N., Armanini, F., Beghini, F., Manghi, P., Tett, A., Ghensi, P., Collado, M.C., Rice, B.L., DuLong, C., Morgan, X.C., Golden, C.D., Quince, C., Huttenhower, C., Segata, N., 2019. Extensive Unexplored Human

- Microbiome Diversity Revealed by Over 150,000 Genomes from Metagenomes Spanning Age, Geography, and Lifestyle. *Cell* 176, 649-662.e20. <https://doi.org/10.1016/j.cell.2019.01.001>
- Pennacchia, C., Ercolini, D., Villani, F., 2011. Spoilage-related microbiota associated with chilled beef stored in air or vacuum pack. *Food Microbiol* 28, 84–93. <https://doi.org/10.1016/j.fm.2010.08.010>
- Pires, D.P., Melo, L.D.R., Azeredo, J., 2021. Understanding the Complex Phage-Host Interactions in Biofilm Communities. *Annual Review of Virology* 8, 73–94. <https://doi.org/10.1146/annurev-virology-091919-074222>
- Poore, J., Nemecek, T., 2018. Reducing food's environmental impacts through producers and consumers. *Science* 360, 987–992. <https://doi.org/10.1126/science.aaq0216>
- Pothakos, V., Devlieghere, F., Villani, F., Björkroth, J., Ercolini, D., 2015. Lactic acid bacteria and their controversial role in fresh meat spoilage. *Meat Science*, 61st International Congress of Meat science and Technology (61st ICoMST), 23-28 August 2015, Clermont Ferrand, France 109, 66–74. <https://doi.org/10.1016/j.meatsci.2015.04.014>
- Quijada, N.M., Dzieciol, M., Schmitz-Esser, S., Wagner, M., Selberherr, E., 2022. Metatranscriptomic Analyses Unravel Dynamic Changes in the Microbial and Metabolic Transcriptional Profiles in Artisanal Austrian Hard-Cheeses During Ripening. *Front Microbiol* 13, 813480. <https://doi.org/10.3389/fmicb.2022.813480>
- Ramanathan, R., Lambert, L.H., Nair, M.N., Morgan, B., Feuz, R., Mafi, G., Pfeiffer, M., 2022. Economic Loss, Amount of Beef Discarded, Natural Resources Wastage, and Environmental Impact Due to Beef Discoloration. *Meat and Muscle Biology* 6. <https://doi.org/10.22175/mmb.13218>
- Roch, F.-F., Dzieciol, M., Strachan, C.R., Chaughtai, M.S., Quijada, N.M., Movsesijan, T., Selberherr, E., 2025. Microbial succession and interaction in vacuum-packed beef: a longitudinal study of bacterial and fungal dynamics. *npj Sci Food* 9, 105. <https://doi.org/10.1038/s41538-025-00479-8>
- Schmieder, R., Edwards, R., 2011. Quality control and preprocessing of metagenomic datasets. *Bioinformatics* 27, 863–864. <https://doi.org/10.1093/bioinformatics/btr026>
- Sequino, G., Cobo-Diaz, J.F., Valentino, V., Tassou, C., Volpe, S., Torrieri, E., Nychas, G.-J., Álvarez Ordóñez, A., Ercolini, D., De Filippis, F., 2025. Environmental microbiome mapping in poultry processing chain and assessment of microbial dynamics in response to different storage conditions. *Food Microbiology* 128, 104734. <https://doi.org/10.1016/j.fm.2025.104734>

- Sequino, G., Cobo-Díaz, J.F., Valentino, V., Tassou, C., Volpe, S., Torrieri, E., Nychas, G.-J., Álvarez Ordóñez, A., Ercolini, D., De Filippis, F., 2024. Microbiome mapping in beef processing reveals safety-relevant variations in microbial diversity and genomic features. *Food Res Int* 186, 114318. <https://doi.org/10.1016/j.foodres.2024.114318>
- Shang, J., Peng, C., Liao, H., Tang, X., Sun, Y., 2023. PhaBOX: a web server for identifying and characterizing phage contigs in metagenomic data. *Bioinformatics Advances* 3, vbad101. <https://doi.org/10.1093/bioadv/vbad101>
- Silveira, C.B., Rohwer, F.L., 2016. Piggyback-the-Winner in host-associated microbial communities. *npj Biofilms Microbiomes* 2, 16010. <https://doi.org/10.1038/npjbiofilms.2016.10>
- Stupar, J., Hoel, S., Strømseth, S., Lerfall, J., Rustad, T., Jakobsen, A.N., 2023. Selection of lactic acid bacteria for biopreservation of salmon products applying processing-dependent growth kinetic parameters and antimicrobial mechanisms. *Heliyon* 9, e19887. <https://doi.org/10.1016/j.heliyon.2023.e19887>
- Sui, L., Yi, Z., Xiao, X., Ma, J., Huang, H., Xu, C., Wang, W., Qu, X., 2023. Investigation of microbial communities across swine slaughter stages and disinfection efficacy assessment in a pig slaughterhouse. *LWT* 187, 115334. <https://doi.org/10.1016/j.lwt.2023.115334>
- Thingstad, T.F., 2000. Elements of a theory for the mechanisms controlling abundance, diversity, and biogeochemical role of lytic bacterial viruses in aquatic systems. *Limnology and Oceanography* 45, 1320–1328. <https://doi.org/10.4319/lo.2000.45.6.1320>
- Valentino, V., De Filippis, F., Sequino, G., Ercolini, D., 2023. Psychrotrophic Bacteria Equipped with Virulence and Colonization Traits Populate the Ice Cream Manufacturing Environment. *Applied and Environmental Microbiology* 89, e00765-23. <https://doi.org/10.1128/aem.00765-23>
- Valentino, V., Sequino, G., Cobo-Díaz, J.F., Álvarez-Ordóñez, A., De Filippis, F., Ercolini, D., 2022. Evidence of virulence and antibiotic resistance genes from the microbiome mapping in minimally processed vegetables producing facilities. *Food Research International* 162, 112202. <https://doi.org/10.1016/j.foodres.2022.112202>
- vegan, n.d. R package for community ecologists: popular ordination methods, ecological null models & diversity analysis. URL <https://github.com/vegandevs/vegan> (accessed 10.25.24).

- Véghová, A., Minarovičová, J., Koreňová, J., Drahovská, H., Kaclíková, E., 2017. Prevalence and tracing of persistent *Listeria monocytogenes* strains in meat processing facility production chain. *Journal of Food Safety* 37, e12315. <https://doi.org/10.1111/jfs.12315>
- Voigt, E., Rall, B.C., Chatzinotas, A., Brose, U., Rosenbaum, B., 2021. Phage strategies facilitate bacterial coexistence under environmental variability. *PeerJ* 9, e12194. <https://doi.org/10.7717/peerj.12194>
- Wang, R., Guragain, M., Chitlapilly Dass, S., Palanisamy, V., Bosilevac, J.M., 2024. Impact of intense sanitization on environmental biofilm communities and the survival of *Salmonella enterica* at a beef processing plant. *Front Microbiol* 15, 1338600. <https://doi.org/10.3389/fmicb.2024.1338600>
- Werum, V., Ehrmann, M., Vogel, R., Hilgarth, M., 2022. Comparative genome analysis, predicted lifestyle and antimicrobial strategies of *Lactococcus carnosus* and *Lactococcus paracarnosus* isolated from meat. *Microbiological Research* 258, 126982. <https://doi.org/10.1016/j.micres.2022.126982>
- Wheatley, P., Giotis, E.S., McKevitt, A.I., 2014. Effects of slaughtering operations on carcass contamination in an Irish pork production plant. *Ir Vet J* 67, 1. <https://doi.org/10.1186/2046-0481-67-1>
- Wood, D.E., Lu, J., Langmead, B., 2019. Improved metagenomic analysis with Kraken 2. *Genome Biol* 20, 257. <https://doi.org/10.1186/s13059-019-1891-0>
- World Wildlife Fund, 2021. Driven to Waste Global Food Loss on Farms [WWW Document]. World Wildlife Fund; Driven to Waste: The Global Impact of Food Loss and Waste on Farms. WWF. URL https://wwf.panda.org/discover/our_focus/food_practice/food_loss_and_waste/driven_to_waste_global_food_loss_on_farms (accessed 10.14.25).
- Yeom, J., Bae, D., Kim, S.A., 2024. Microbial dynamics of South Korean beef and surroundings along the supply chain based on high-throughput sequencing. *Meat Science* 214, 109520. <https://doi.org/10.1016/j.meatsci.2024.109520>
- Yu, Q., Gu, X., Liu, Q., Wen, R., Sun, C., 2024. Effect of wet-aging on meat quality and exudate metabolome changes in different beef muscles. *Food Research International* 184, 114260. <https://doi.org/10.1016/j.foodres.2024.114260>
- Zhang, T., Zhang, W., Ai, D., Zhang, R., Lu, Q., Luo, Q., Shao, H., 2018. Prevalence and characterization of *Clostridium perfringens* in broiler chickens and retail chicken meat in central China. *Anaerobe, MALDI-TOF MS in anaerobe diagnostics* 54, 100–103. <https://doi.org/10.1016/j.anaerobe.2018.08.007>

- Zwirzitz, B., Wetzels, S.U., Dixon, E.D., Fleischmann, S., Selberherr, E., Thalguter, S., Quijada, N.M., Dzieciol, M., Wagner, M., Stessl, B., 2021. Co-Occurrence of *Listeria* spp. and Spoilage Associated Microbiota During Meat Processing Due to Cross-Contamination Events. *Front. Microbiol.* 12. <https://doi.org/10.3389/fmicb.2021.632935>
- Zwirzitz, B., Wetzels, S.U., Dixon, E.D., Stessl, B., Zaiser, A., Rabanser, I., Thalguter, S., Pinior, B., Roch, F.-F., Strachan, C., Zanghellini, J., Dzieciol, M., Wagner, M., Selberherr, E., 2020. The sources and transmission routes of microbial populations throughout a meat processing facility. *npj Biofilms Microbiomes* 6, 26. <https://doi.org/10.1038/s41522-020-0136-z>

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