



Cold storage, hot spots: Household refrigerators as under-recognized hubs of microbial diversity

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ABSTRACT

Household refrigerators are indispensable for food storage but also serve as reservoirs for diverse microbial communities, including potential pathogens. International guidelines recommend storage below 5 °C but give little guidance on domestic refrigerator hygiene. This study investigates how temperature and hygiene shape microbial burden, antimicrobial resistance, and community structure in domestic refrigerators using shotgun metagenomic sequencing at species-level resolution. Swabs from 45 refrigerators revealed substantial diversity, with *Acinetobacter*, *Pseudomonas*, and *Staphylococcus* as dominant genera. Only 38% of refrigerators maintained temperatures below 5 °C, while 24% exceeded 7 °C. Cleaning frequency was the key factor associated with bacterial cell equivalents (BCE) and diversity, with high loads linked to infrequent cleaning. Pathogenic species, including *Bacillus cereus* and *Staphylococcus aureus*, were detected in 60% of refrigerators; *Listeria monocytogenes* and *Yersinia intermedia* occurred sporadically. Resistome analysis identified antimicrobial resistance genes (AMRG), including bla_{OXA} variants, particularly in older appliances, indicating a potential domestic reservoir of resistance. The findings underscore the need for clearer household recommendations on refrigerator hygiene and temperature control, and advance our understanding of the microbial communities occurring in household refrigerators and its implications for consumer health, thereby contributing to improved domestic food safety.

1. Introduction

Household refrigerators are essential for food storage and preservation, yet they may also provide an environmental niche for the growth and persistence of diverse microbial communities that can be detrimental to the food products and human health. Previous studies have shown that consumers often fail to recognize the critical impact of proper hygiene and temperature management on personal health in kitchen and refrigerator settings, thereby representing a weak point in the cold chain (Catellani et al., 2014; Dumitraşcu et al., 2020; Terpstra et al., 2005).

International health authorities, including the World Health Organization (WHO), European Food Safety Authority (EFSA), and Food and Drug Administration (FDA), specifically recommend maintaining refrigerator temperatures below 4–5 °C to limit the growth of key foodborne pathogens such as *Listeria monocytogenes*, *Salmonella* spp., and

pathogenic *Escherichia coli* (U.S. Food and Drug Administration, 2023). This recommendation is particularly relevant for non-shelf-stable foods (i.e., those with a pH above 4 and water activity above 0.88) (Koutsoumanis et al., 2020; WHO, 2022). On the other hand, these health authorities have not published specific recommendations on cleaning schedules for domestic refrigerators, in contrast to long-standing efforts to educate the public about the need to clean kitchen surfaces due to the risks of cross-contamination (U.S. Food and Drug Administration, 2023; WHO, 2022). Although the presence of pathogens in household refrigerators has been studied for decades (Jackson et al., 2007), exploring the whole microbiome represents a more integrative approach, as nonpathogenic organisms can contribute to overall microbial persistence via mechanisms such as biofilms and lead to community-level resistance to conventional cleaning methods (Voglauer et al., 2025; Zwirzitz et al., 2021).

Using metagenomic analysis, we sought to explore how either

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temperature or cleaning frequency affects microbial composition within household refrigerators. We categorized two potentially distinct microbial niches based on temperature: i) the cooler zone (below 5 °C), and ii) the warmer zone (above 7 °C) based on the recommendations and warnings from health authorities, with a transitional zone of 5–7 °C representing the majority of refrigerators of Western consumers (Dumitraşcu et al., 2020). A temperature-dependent niche with increased numbers of pathogens in the warmer zone would provide evidence supporting the effectiveness of recommended refrigeration temperatures in enhancing food safety. Furthermore, we collected data on the frequency and thoroughness of refrigerator cleaning to determine whether either influences microbiome composition, potentially enabling differentiation between mature and immature microbial communities based on the time elapsed since the last reported cleaning.

Many studies have been conducted over recent decades focusing on different aspects of refrigerator-associated microbiota such as effects of temperature (Andritsos et al., 2021; Dumitraşcu et al., 2020; Evans & Redmond, 2016; Janjić et al., 2016; Mori et al., 2020), household demographics (Düven et al., 2021; Vegara et al., 2014), appliance models (Jovanovic et al., 2022), cleaning methods and frequency (Macías-Rodríguez et al., 2013; Mori et al., 2020; Vegara et al., 2014), as well as fill level, food placement and different compartments (Jofré et al., 2019; Mori et al., 2020), using a variety of culture-dependent microbiological and molecular biology methods as well as metataxonomic. The hygienic status of the refrigerator is associated with different factors, including socio-demographic conditions, where elderly people in rural areas demonstrate the lowest awareness (Carstens et al., 2022; Dumitraşcu et al., 2020; Düven et al., 2021; Jovanovic et al., 2022; Sinclair & Gerba, 2011; Vegara et al., 2014).

Temperature monitoring is a critical component of ensuring hygienic conditions in household refrigerators. The measurement of refrigerator temperatures range from single-point readings to continuous, week-long profiling using multiple sensors per unit. Average refrigerator temperatures frequently exceed the recommended 5 °C because many domestic units lack built-in thermometers, and, even when thermometers are installed, households are not always aware of or interested in the recommended settings (Andritsos et al., 2021; Derens-Bertheau et al., 2015; Evans & Redmond, 2016; Janjić et al., 2016; Jofré et al., 2019). Conventional microbiological studies in refrigerators typically rely on Total Viable Count (TVC), Total Coliform Count (TCC), and the selective cultivation of certain potential pathogens, including *L. monocytogenes*, *E. coli*, *Staphylococcus* spp. and *Bacillus cereus*, even though they are scarcely detected (Catellani et al., 2014; Düven et al., 2021; Jackson et al., 2007; Macías-Rodríguez et al., 2013; Ye et al., 2019). Of particular relevance is the presence of causative agents, which EFSA defines as the following: “the pathogen or its products, such as a toxin or bioactive amine, considered to be the cause of the food-borne outbreak” (European Food Safety Authority, 2014).

The use of metataxonomic approaches involving culture-independent 16S rRNA gene/ITS amplicon sequencing has deepened the microbial composition of general household/kitchens and pointed out *Pseudomonas*, *Bacillus*, *Staphylococcus*, *Pantoea*, *Saccharomyces*, *Candida*, and *Penicillium*, as the most ubiquitous bacteria and fungi (Jeon et al., 2013; Ye et al., 2019). Gaining deeper insights into the composition and dynamics of microbial communities in domestic refrigeration systems is necessary for developing effective strategies to mitigate hygienic risks and enhance food safety at the consumer level. The main objectives of this study were to (i) characterize microbial communities in domestic refrigerators at species-level resolution using shotgun metagenomic sequencing, (ii) determine how compliance with recommended refrigeration temperatures (≤ 5 °C) and household hygiene practices (including cleaning frequency) relate to microbial community structure and the distribution of antimicrobial resistance genes and (iii) quantify microbial burden and profile antimicrobial resistance genes. Collectively, these analyses aim to inform clearer household guidance on refrigerator hygiene and temperature control and to advance

understanding of the refrigerator microbiome and its implications for consumer health and domestic food safety.

2. Materials and methods

2.1. Sampling

Samples were obtained from households of high school students of two partner schools in Austria, as part of the “Micro-Tramper” Sparkling Science 2.0 OeAD (Agentur für Bildung und Internationalisierung) project, as well as from volunteering members of the Veterinary University of Vienna and their family members, forming three distinct geographical cohorts. All participants had at least basic knowledge in microbiology and received additional training to ensure standardization of the sampling process. This included a detailed instruction leaflet and an online presentation outlining each step of the workflow. Each sampling kit included a ThermoPro TP516EU Digital Meat Thermometer (iTronics Company Limited, China), a SampleRight™ High Density Polyurethan sponge with HiCap™ Neutralizing Broth (World Bio-products LLC, USA), a pair of nitrile gloves, and the instruction leaflet. Participants were asked to record metadata, including refrigerator age, model, manufacturer, fill level, number of household members, presence of pets, time since last cleaning, cleaning frequency per year, sampled area, and items stored in the refrigerator at the time of sampling. To minimize variation in participant interpretation, “cleaning” was operationally specified as wiping the entire sampled shelf with a wet cloth or sponge, with or without the use of soap or disinfectants. Soaking up spilt liquids like dairy or soda using a dry paper towel or similar was specified as not to be included when counting the number of cleanings per year and date of the last cleaning.

One day prior to sampling, participants were instructed to place a glass filled with tap water at the back of the designated sampling shelf to allow the contents to reach thermal equilibrium before temperature measurement. Temperature measurements were performed in triplicate immediately before surface sampling. All objects on the designated shelf were removed and the entire surface of the shelf was sampled while wearing the provided gloves. The surface was wiped from front to back for 15 s using one side of the sponge, followed by left to right for 15 s using the opposite side of the sponge. The sponge was then immediately back into the plastic bag, which was then sealed and stored overnight in the refrigerator. Samples were subsequently transported at ambient temperature (during the winter season) to the project location, handed over to members of the Veterinary University of Vienna, and stored at -21 °C until DNA extraction. A total of 49 samples with sufficient metadata were collected. A processing control (sponge only) was included.

2.2. DNA extraction and sequencing

For the DNA extraction, up to 2 ml sample buffer was manually wrung from each sponge and then processed using the DNeasy® PowerFood® Microbial Kit (QIAGEN, Germany) following the manufacturer's instructions with a final elution in 40 µl RO-H₂O. The extracted DNA was measured using the Qubit™ 1X dsDNA High Sensitivity Assay Kit on a Qubit™ 2.0 Fluorometer (Thermo Fisher Scientific, USA).

The samples were sequenced by Procomure NGS (Berghheim, Austria) using their Deep Shotgun Metagenome Analysis service on an Illumina NovaSeq 6000 with 2×150 bp paired-end run, which achieved a minimum of 36 M and a median of 55 M paired-end reads per sample after FastQC. 45 out of 49 samples passed the sequencing quality control.

2.3. qPCR

To assess the microbial load of the refrigerator shelf as well as total abundance, a quantitative PCR (qPCR) targeting the 16S rRNA gene was

performed using 2 µl of extracted DNA per reaction, in duplicate. The bacterial cell equivalents (BCE) for each sample were determined using the protocol established by Zwirzitz et al. (2019). The assay employed EvaGreen® Dye-based detection chemistry (Biotium, Inc., USA) with primers 341F 5'-CCTACGGGAGGCAGCAG-3' and 543R 5'-ATTACCGCGGCTGCTGG-3' (Muyzer et al., 1993). Total BCE per sample was calculated using a conversion factor of six 16S rRNA gene copies per bacterial cell (Stoddard et al., 2015).

2.4. Bioinformatic analysis of the metagenomic data and statistical analysis

The raw shotgun sequencing reads were quality-controlled to remove low quality and short reads, as well as residual barcodes and adapters, by using Trimmomatic v0.39.2 (Bolger et al., 2014). Taxonomic profiling was performed by subjecting the QC-sequences to the MetaPhlAn v4.1.1 pipeline (Blanco-Míguez et al., 2023).

Total abundance of a taxon was inferred by normalizing the relative abundance of each taxon to the total bacterial cell equivalents (BCE) per sample using the following formula:

$$\text{Total Abundance(Taxon)} = \frac{\text{Relative Abundance(Taxon)}}{100} \times \text{BCE(Sample)}$$

Unclassified reads were determined using the –unclassified_estimation flag from MetaPhlAn 4. Alpha-diversity was quantified in R using the Shannon diversity index (H') with p_i being the proportional abundance of taxon i :

$$H' = - \sum_i p_i \log(p_i)$$

Bray–Curtis distances were used as the basis for inferring beta-diversity, and both Bray–Curtis and PERMANOVA were calculated using `vegdist()` and `adonis2()` from the *vegan* R package. Similarly, pairwise Spearman-based co-occurrence network analysis was performed among the 50 most abundant taxa per dataset, keeping only positive correlations with $\rho \geq 0.5$ and used to construct an undirected adjacency matrix. This matrix was converted into a network graph, and clusters were identified using the `cluster_walktrap()` function from the *igraph* package. Sample-wise Bray–Curtis dissimilarity was computed using `vegdist()`, and Principal Coordinates Analysis (PCoA) was performed via classical multidimensional scaling using the `cmdscale()` function.

Resistome was assessed by using the ResFinder database (downloaded on 2025-04-24) (Bortolaia et al., 2020). In order to prevent short-read alignment to closely related genes, the genes from the ResFinder database were clustered by using CD-HIT v4.8.1 (Li & Godzik, 2006) over 90% coverage and identity, resulting in 740 clusters. Then, resistome profiling was performed by aligning the QC-reads against the clustered-ResFinder database by using `bowtie2` v2.5.4 (Langmead & Salzberg, 2012) and by polishing the alignments using `featureCounts` (Liao et al., 2014) and `SAMtools` (Danecek et al., 2021).

Counts per million reads (CPM) of the antimicrobial resistance genes (AMRG) were calculated by applying a scaling factor based on the proportion of reads assigned to the bacterial kingdom, as determined by MetaPhlAn, using the following formula:

$$\text{CPM} = \frac{\text{AMRG} \times \text{Bacterial Proportion}}{\text{Total Number of Reads}} \times 10^6$$

Genes were further filtered to include only those with sufficient coverage, defined as a depth ≥ 5 and breadth ≥ 0.8 .

All R packages used in downstream analysis are listed in Supplemental Table 1.

2.5. Metadata availability

Refrigerator models, temperature readings, household

demographics, refrigerator status and presence of specific food items reported by participating are listed in Supplemental Table 2.

3. Results

3.1. Survey and temperature profiles with widely varying BCE loads and no detected parameter associations

The results from the questionnaire completed by participants are summarized in Fig. 1, which shows that the average household consisted of mid-sized families (four members) with one pet, and refrigerators with a median age of 9 years (although many units were older than 15 years). Most participants reported storing primarily dairy products (i.e., butter, milk, cheese and yoghurt), along with other central European staples like jams and sausages. No households reported storing seafood or raw meat, and none followed a vegan diet. Only 17 out of 45 refrigerators maintained temperatures below 5 °C as recommended by the WHO, while 11 units exceeded 7 °C (Supplemental Figure 1 (A)), which represents a worst-case storage temperature used in shelf-life studies for manufacture to retail display case (EFSA, 2020). However, cleaning practices seemed to be stricter in households with higher refrigeration temperatures (Supplemental Figure 1 (B)).

BCE values obtained from shelf swab samples ranged from 1.56×10^4 to 1.27×10^9 , with the median of 1.86×10^6 (per sample). No significant correlations were observed between BCE and the other measured parameters (Supplemental Fig. 2 and Supplemental Table 3). With a median sampling surface area of 1215 cm², the BCE per cm² ranged from 6.22×10^5 to 9.84×10^5 , with a median of 1413 BCE/cm².

3.2. High microbial counts correspond with low species richness

A total of 2184 microbial species were identified across all samples, with individual sample richness ranging from 26 species (sample G2) to 589 species (sample F1). On average, low-temperature refrigerators harboured 224.4 species, compared to 250.1 species in high-temperature units. When stratified by BCE quartiles, refrigerators in the first quartile (lowest BCE) contained an average of 259.6 species, whereas those in the fourth quartile (highest BCE) hosted only 93.6 species (Supplemental Table 4).

In order to assess the alpha-diversity, we calculated Shannon indices, which ranged from 0.25 to 4.13 with no significant difference between samples below 5 °C, above 7 °C, or with temperature values in-between (Fig. 2 (A) and (B)). However, significant differences were observed comparing Shannon values between the different BCE quartiles, with Q1 BCE samples having twice the Shannon index of Q4 BCE samples (3.2 vs 1.6 for species).

Beta-diversity was assessed by calculating Bray–Curtis dissimilarity and conducting a PCoA in Fig. 2 (C): data points are coloured based on co-occurrence clusters, which were defined by Spearman correlations ≥ 0.5 among the top 50 taxa, converted to an adjacency graph, and clustered; samples inherit the cluster of their dominant taxon. A PERMANOVA performed on the Bray–Curtis dissimilarity matrix revealed statistically significant effects only for the variables ‘time since last cleaning’ and ‘sampling location’ (Fig. 3).

3.3. Acinetobacter, psychrophilic and spoilage-associated microbes dominated the refrigerator microbiome

The relative abundances of the most common species are shown in Fig. 4. *Acinetobacter* was the most abundant genera overall (12.7% relative abundance overall) followed by *Pseudomonas* (4.8%), *Psychrobacter* (3.7%), *Moraxella* (3.7%), *Brochothrix* (2.7%), *Leuconostoc* (2.6%), *Microbacterium* (2.4%), *Streptococcus* (2.1%), *Staphylococcus* (1.8%), *Epilithonimonas* (1.6%), *Debaryomyces* (1.5%), *Brevibacterium* (1.2%), *Kocuria* (1.1%), *Latilactobacillus* (1.1%), *Corynebacterium* (1.1%) and *Carnobacterium* (1.1%) (see Supplemental Table 5).

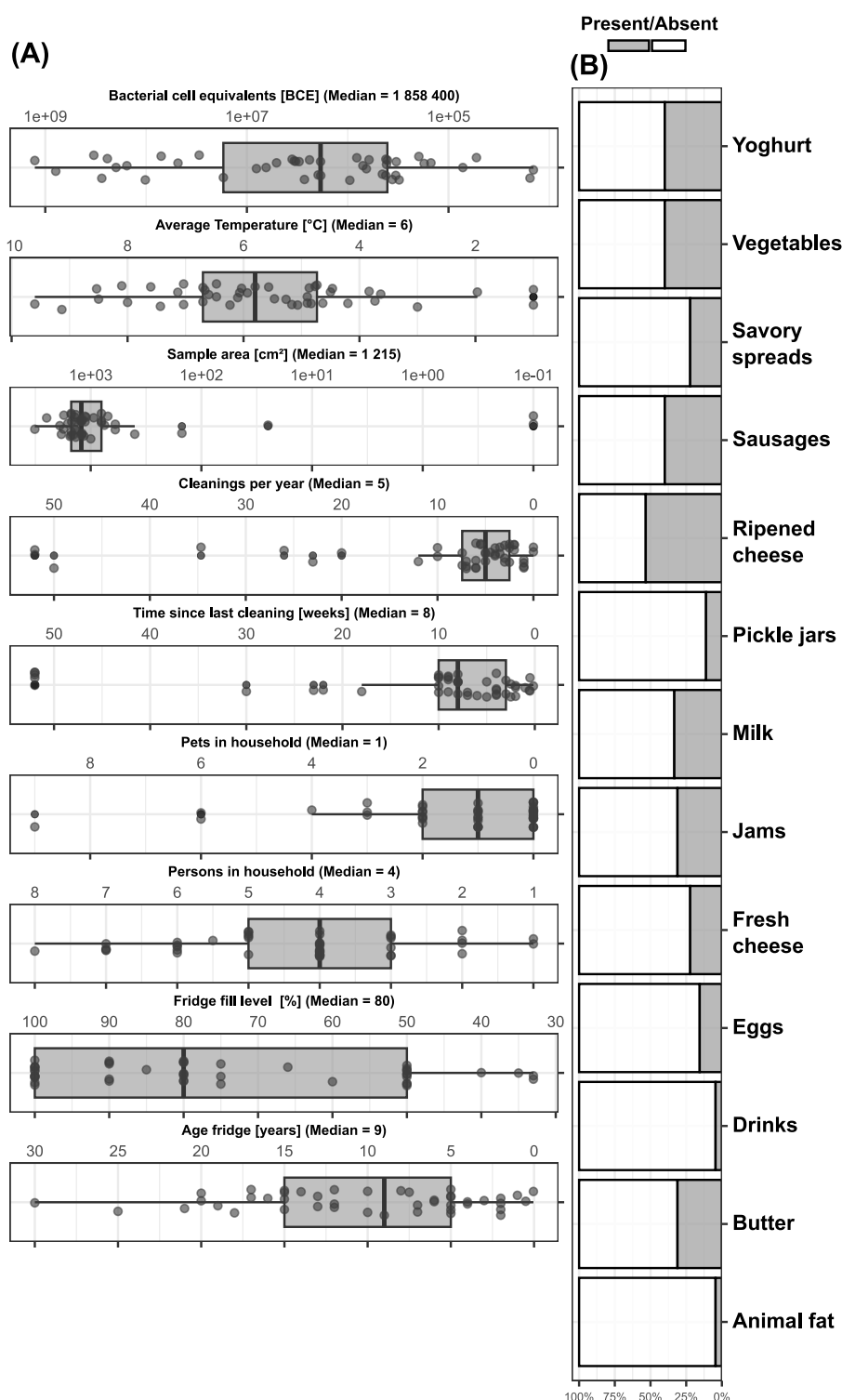


Fig. 1. Results from the questionnaire and refrigerator swab sample analysis. (A) Box plots showing BCE per sample values obtained via qPCR, refrigerator temperature readings, household demographics, and refrigerator status of participating families. (B) Bar plots depicting the relative presence of specific food items reported by participants at the time of sampling.

Acinetobacter is mostly comprised of three different species, *A. johnsonii*, *A. lwoffii* and *A. pittii* that make up 87.3% of all reads assigned to *Acinetobacter*. The list of the 50 most abundant species encompasses 33 genera, of which *Pseudomonas* has the highest number present (*P. lactis*, *P. carnis*, *P. reactans*, *P. veronii*, *P. fragi*, *P. fluorescens*) while more than 99.99% of all reads assigned to genus *Moraxella* come

from a single species genomic bin (SGB ID: SGB10439).

Out of the 45 refrigerators sampled, 27 contained at least one of the pathogens listed by the EFSA. Among these, *Bacillus cereus*, commonly associated with flour, was the most abundant detected in 23 samples and reaching a relative abundance of 5.72% in samples with high BCE levels (D3, B6). *Brucella* was identified in 14 samples, which collectively

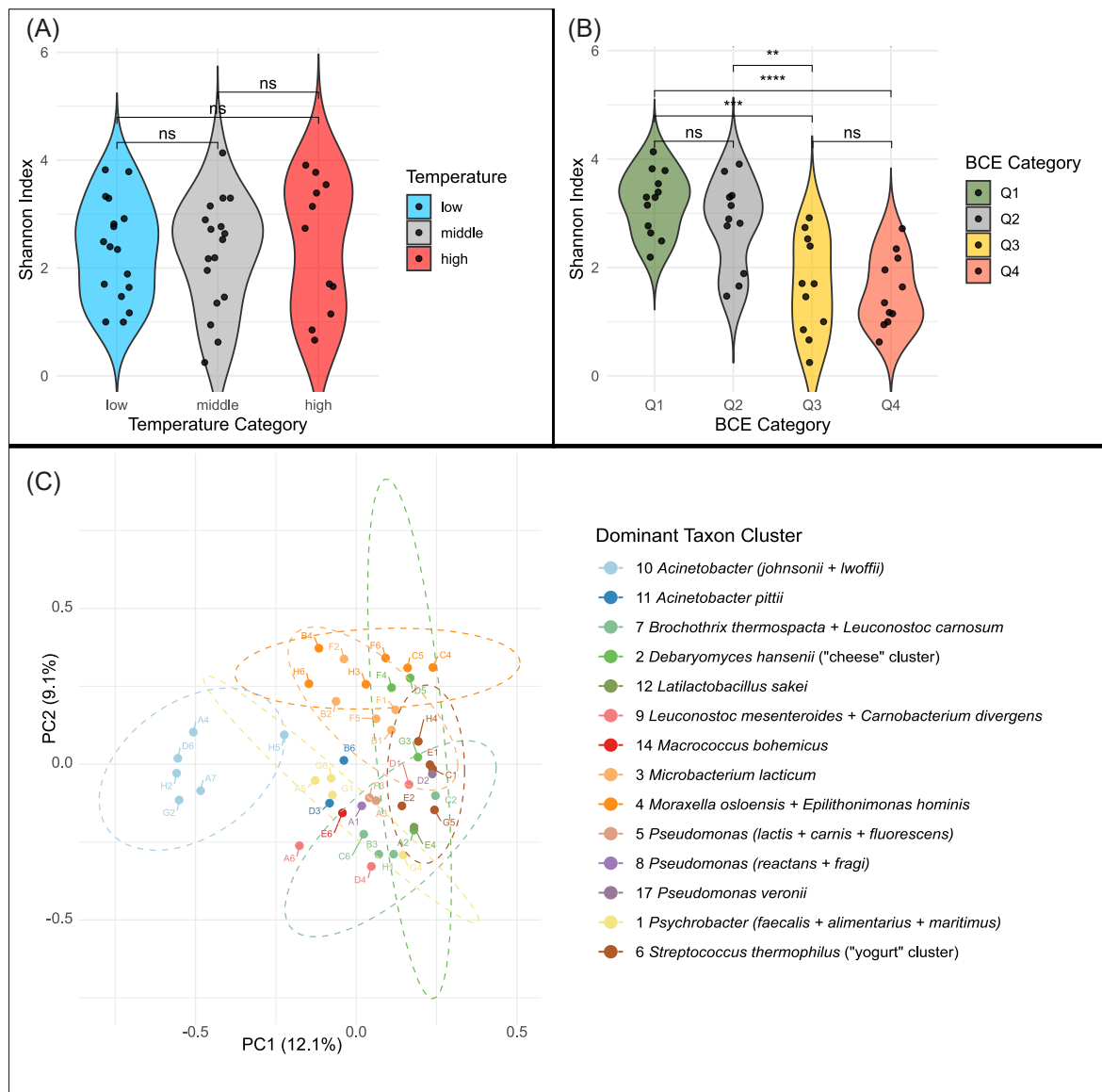


Fig. 2. Volcano plots comparing Shannon diversity indices of refrigerator swab samples. (A) Comparison between different refrigerator temperature groups ($<5^{\circ}\text{C}$ for low, between 5°C and 7°C for middle, $>7^{\circ}\text{C}$ for high). (B) Comparison between samples from different BCE value per sample quartiles. (C) Beta diversity PCoA based on the Bray-Curtis dissimilarity of species-level relative abundances, coloured by co-occurrence cluster affiliation.

comprised reads assigned to *B. thiophenivorans*, *B. pituitosa*, *B. anthropi*, *B. haematophila*, *B. pseudogrigonensis*, and *B. rhizosphaerae*, each found in 1–7 samples. *Staphylococcus aureus* was the next most common causative pathogen, with a single SGB detected in 7 samples. *Yersinia intermedia*, *Campylobacter ureolyticus*, *Mycobacterium numidiamassiliense*, and *Listeria monocytogenes* were each detected in one sample.

Among fungi, *Debaryomyces hansenii* was the most abundant and prevalent, with an overall relative abundance of 1.5% (ranging up to 26.49% in sample A2) and was detected in 36 samples. *Saccharomyces cerevisiae* was detected in 31 samples, with a maximum relative abundance of 6.26%. Other fungal taxa included *Pichia* (*P. kluyveri*, *P. kudriavzevii*, *P. fermentans* and *P. membranifaciens*), present in 6 samples, *Penicillium* (*P. italicum*, *P. roqueforti*, *P. chrysogenum*, *P. camemberti*, among others), present in 9 samples, *Malassezia restricta*, was found in 16 samples and various species formerly assigned to *Candida*, such as *C. sake*, *C. psychrophila* and *C. intermedia*, as well as current members of the genus *Candida*, were also present. Archaea were detected in 12 samples, including unclassified members of *Nitrososphaerales*, *Methanobacteriaceae* and *Methanosarcina*.

3.4. Higher resistome loads were found in older refrigerators

A Pearson correlation coefficient was calculated for the measured parameters, revealing a statistically significant, moderately positive association ($R^2 = 0.41$, $p = 0.005$) between the total CPM of resistance genes and refrigerator age. Notably, all samples with total CPM values exceeding 100 originated from refrigerators that were at least 12 years old at the time of sampling, whereas the reported time since the last cleaning shows a negative correlation ($R^2 = -0.29$, $p = 0.056$). A moderately positive correlation ($R^2 = 0.44$, $p = 0.003$) assigned to the presence of drinks on the sampling location should be discarded due to the low number of positive samples ($n = 2$) (see [Supplemental Table 6](#)). Sample groups characterized by low total CPM included those associated with cluster 7 (*Brochothrix thermospacta* cluster), cluster 6 (*Streptococcus thermophilus* "Yoghurt" cluster), and cluster 2 (*Debaryomyces hansenii* "cheese" cluster).

High resistome loads were mostly associated with *Acinetobacter*-dominated samples. Samples associated with cluster 10 (*Acinetobacter johnsonii* cluster) exhibited high CPM across all samples (see [Fig. 5](#)),

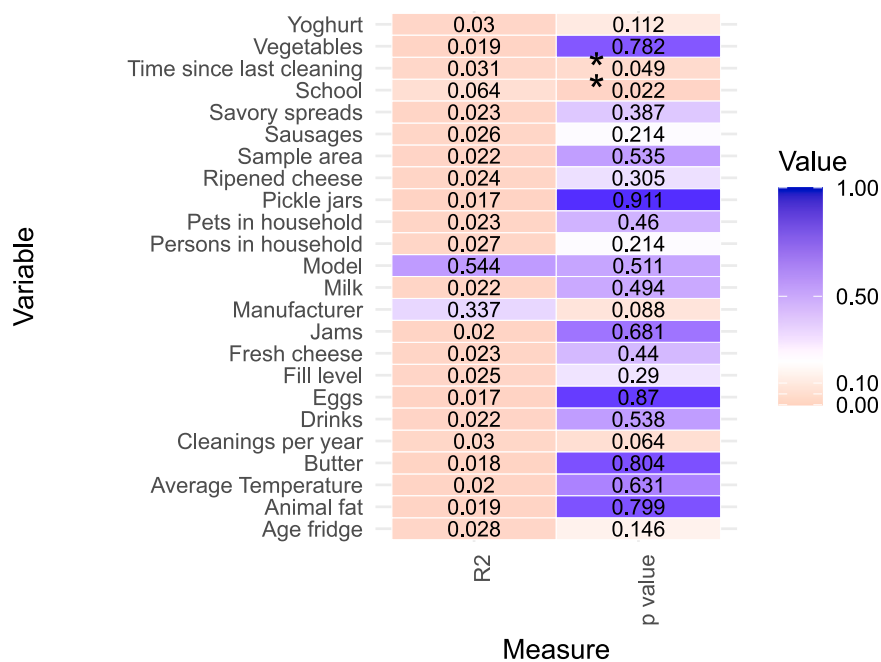


Fig. 3. PERMANOVA heatmap displaying r^2 and p values for species-level relative abundances across metadata variables, based on the Bray-Curtis distance matrix.

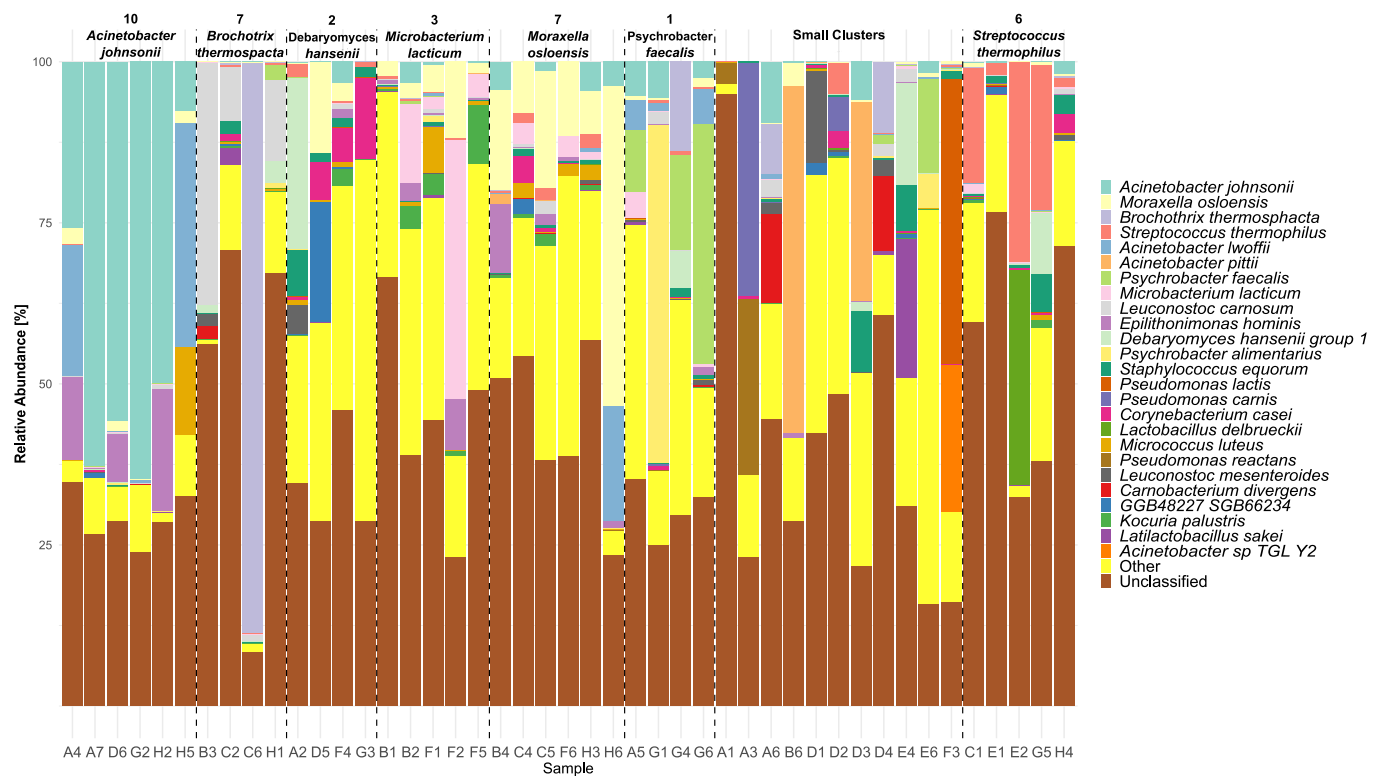


Fig. 4. Bar plots displaying relative abundances of species of each refrigerator swab sample, grouped by co-occurrence cluster affiliation.

containing the AMRG blaOXA-280. Samples A4 and H5, dominated by *Acinetobacter lwoffii* (also associated with cluster 10), contained the closely related blaOXA-496. Other high-abundance *Acinetobacter* samples, B6 and D3, which were dominated by *Acinetobacter pittii*, also showed high total CPM, with blaOXA-417 and blaADC-25 accounting for

the majority of resistance genes. Additional resistance genes with high CPM values detected across multiple samples included aph(6)-Id, aph(3'')-Ib, sul(2), msr(E), erm(C) and tet(39). Low levels of polymyxin resistance genes (mcr-4/5/7/9/10), were detected, with CPM values up to 0.6. No vancomycin resistance genes were shown.

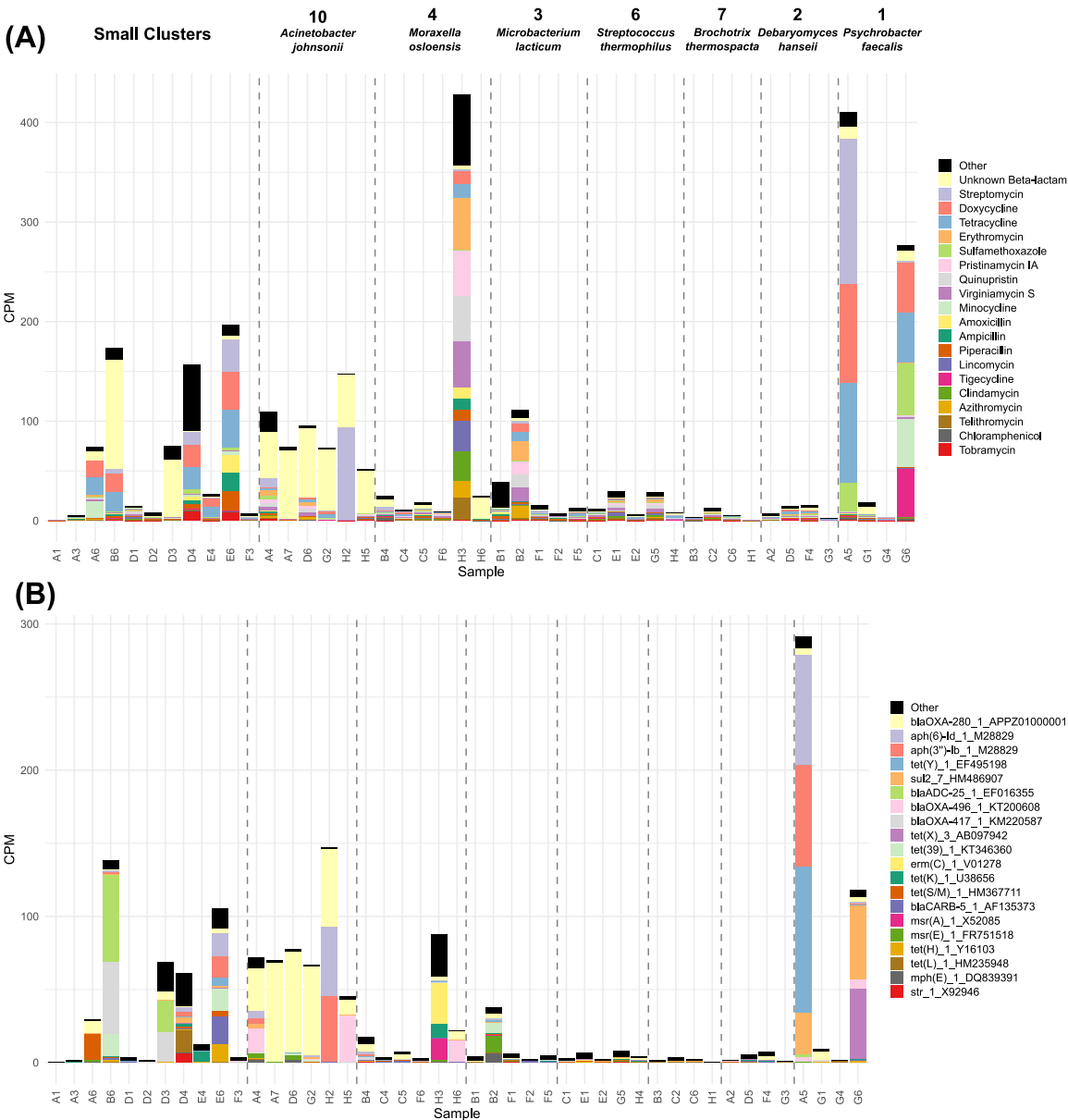


Fig. 5. Counts per million (CPM) of reads assigned to ResFinder for the 20 most abundant: (A) Resistance phenotypes and (B) Resistance genes. Each refrigerator swab sample is grouped according to its affiliation with a co-occurrence cluster.

4. Discussion

The results of this study expand our understanding of microbial communities inhabiting domestic refrigerator surfaces, particularly regarding the effects of temperature and cleaning regimens. Previous assessments of the refrigerator microbiome have primarily relied on culture-based and/or 16S rRNA gene amplicon surveys. In contrast, our methodology employed shotgun metagenomic sequencing, enabling not only the identification of microbial taxa but also the characterization of the antimicrobial resistance profile, which provides key insights for public health officials and remains poorly understood outside clinical contexts. Our participant cohort was largely drawn from households of pupils participating in the “Micro-Tramper” project, which tends to include middle-class families from medium-to-large cities (per OECD-EC definitions). However, these schools have large catchment areas, and many participants also came from the surrounding rural regions, resulting in the inclusion of multi-generational homes and representation of older adults. University-affiliated participants further

contributed single/bachelor households and urban Vienna residences, where grocery shopping patterns differ from those of rural homes. Although this recruitment strategy may not perfectly reflect the general population, we observed no systematic bias in temperature readings or bacterial cell equivalent (BCE) counts across demographic groups, suggesting that our principal microbiological findings are robust across the range of household types included.

Our shotgun results broadly align with prior household/kitchen surveys reporting psychrotolerant, food- and skin-associated taxa; the absence of *Enhydrobacter* in our dataset likely reflects the specific shelf type sampled rather than a true absence from domestic environments (Klein-Jöbstl et al., 2019; Staley et al., 1987). Consistent with Ye et al. (2019), we observed frequent *Bacillus*, *Acinetobacter*, *Pseudomonas*, and common yeasts such as *Saccharomyces*; species-level resolution clarified that our *Acinetobacter* signals were dominated by skin/food-associated taxa rather than *A. baumannii*, a pathogen commonly found in hospitals. Genera including *Enterococcus* and *Citrobacter* were less prominent in our cohort. As for fungi, Ye et al. (2019) detected the ITS sequences of

Saccharomyces cerevisiae in 75% of their samples, approximately matching our 31 out of 45 positive samples (69%) as well as several *Candida* species. While we did not identify any *Schwanniomycetes* or *Kazachstania*, we detected several *Penicillium* species, possibly attributable to either ripened cheese or fruit rot, which is corroborated by the questionnaire results.

Culture-based surveys (e.g., Catellani et al., 2014) reported lower recoveries of psychrotolerant taxa than our metagenomic profiling, while our data indicate broad *Pseudomonas* ubiquity with varied species-level composition. Considering the BCE/cm² results from our qPCR analysis, while they are not directly comparable to the CFU/cm² (Reichert-Schwillinsky et al., 2009), 41 out of 45 of refrigerators exceed 80 BCE/cm², surpassing the corresponding “practical target of acceptability” of 80 CFU/cm² set by the Public Health Laboratory Service (PHLS) in the UK (Herbert et al., 1990).

Our results corroborate prior research regarding the large presence of human skin-associated taxa on the genus level, but the higher taxonomic resolution of shotgun sequencing shows the high species richness of many dominant genera like *Pseudomonas*, *Microbacterium*, or *Corynebacterium*, while other dominant genera like *Moraxella*, *Brochotrix* or *Debaryomyces* are represented with a few or just a single species. The species level resolution also allowed us to identify many taxa related to fermented food, like *Brevibacterium aurantiacum* (surface ripened cheese), *Leuconostoc mesenteroides* (sauerkraut, kimchi), *Leuconostoc carnosum* or *Latilactobacillus sakei* (surface ripened sausages) which indicates that direct contact between the surface of food and the shelf/hands is prevalent and combined with the self-reported behaviour of shuffling items between compartments for convenience (Vegara et al., 2014), cross contamination between these surface ripened foods, vegetables, fruits and other products deposited directly on the shelves are likely. Although classic mesophilic pathogens were uncommon, we detected causative agents such as *Bacillus cereus* and *Staphylococcus aureus* on nontrivial prevalence, suggesting that cleaning intervals and handling practices likely influence pathogen persistence more than temperature alone. The median of 35.1% of unclassified reads (ranging from 8.4% to 94.9%) is consistent with similar sample origins (Wright et al., 2023).

Results from the PERMANOVA (Fig. 3) are consistent with the observation of Mori et al. (2020) that cleaning frequency, or, in our case, the reported time since the last cleaning, appears to have the most significant, albeit modest, impact on the microbiome. While we could not establish a correlation (Spearman) between the BCE values and either of our cleaning metadata (Supplemental Table 2), beta diversity was affected by it, and the slight mismatch in significance between the last cleaning and the reported number of cleanings per year might be explained by the former being easier to remember while the latter is more easily overestimated. The cleaning frequencies between the two studies are comparable: 17% of Japanese refrigerators were cleaned more than once per month, compared with 8 of 45 (18%) in Austria. The correlation between CPM of resistance genes and the time since last cleaning, as well as the age of the refrigerator, supports our view of potentially insufficient cleaning methods leading to an increasing amount of AMRGs over the lifetime of a refrigerator, possibly also due to the continued build-up of biofilms.

The high prevalence of AMRGs, especially for beta-lactams, tetracyclines and aminoglycosides, suggests that even a cold environment such as a domestic refrigerator could be a reservoir for, or origin of, multidrug-resistant bacteria (MDR). Although the more clinically relevant *Acinetobacter baumannii* was not detected, samples were dominated by members of the genus *Acinetobacter*, which exhibit high levels of resistance to oxacillin-related beta-lactams and are known to transfer AMRGs via mobile elements such as plasmids (Peleg et al., 2008). The presence of these AMRGs and their implied spread via horizontal gene transfer (HGT) further complicates the distinction between “hospital superbugs” and environmental microbiota, thus reinforcing the One-Health approach and perspective.

Aside from potential pathogens, food waste remains the other major concern, with fresh produce being most at risk from environmental spoilers, particularly *Penicillium*, of which we detected both dairy and fruit-associated species in 9 out of 45 samples, with the issue being their potential growth even at temperatures of 4 °C. This further emphasizes the need for cleanliness over simple reliance on low temperatures. Given the variability of cold-chain quality internationally and within individual distribution networks, one must plan for a worst-case holding temperature of 7 °C through to the retail cabinet (Koutsoumanis et al., 2020). Our mean temperature was comparable to several European cohorts, yet many units exceeded recommended thresholds, underscoring persistent gaps in domestic temperature control.

The issue of temperature and food waste via shelf life has also been discussed in the past: Holsteijn and Kemna (2018) address the optimization of available refrigerator space, suggesting than greater granularity through the inclusion of meat chiller/salad chiller compartment at −1 °C and +2 °C, respectively, which could extend the shelf life of these food categories by several weeks. However, they note that compartmental and structural changes to refrigerators might increase energy consumption, negatively affecting EU energy labelling and thereby reducing consumer appeal. Nevertheless, the resulting reduction in food waste could offset the additional energy required to produce that food.

5. Conclusion

The conundrum is multifaceted, but temperature remains crucial for food safety and for reducing food waste through increased shelf life. Advances in technology, legislation, and consumer knowledge can all contribute to improvements in both areas. However, basic hygiene appears to be overlooked, and one of the most vulnerable groups, the elderly, is least likely to adopt either new technology or cleaning habits, often due to affordability or accessibility issues. There is a general misconception that the refrigerator is an inert holding area for food, disproven by the high species richness determined through shotgun sequencing in this study and it should be viewed as an extension of the kitchen worktop or dinner table, requiring regular cleaning both for health and food waste reasons, which is not yet sufficiently addressed by public outreach of international health authorities. Indeed, the authorities should treat the detection of crucial AMRG as a warning sign. Simple improvements, such as using intermediate storage surfaces such as dishwasher-safe bowls, plates, or other containers, could increase cleaning frequency and thus lower the risk of cross-contamination.

CRedit authorship contribution statement

Moritz Hartmann: Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation. **Monika Dzieciol:** Writing – review & editing, Supervision, Methodology, Investigation. **Cameron R. Strachan:** Writing – review & editing, Software, Methodology, Data curation. **Narciso M. Quijada:** Writing – review & editing, Supervision, Software, Methodology, Formal analysis. **Evelyn Selberherr:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Methodology, Funding acquisition, Conceptualization.

Declaration of competing interest

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

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Further information about the “Micro-Tramper” Citizen Science project can found at: <https://oead.at/en/study-research-teaching/citizen-science/sparkling-science/projects/overview/detail/micro-tramper>.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.lwt.2026.119119>.

Data availability

The data for this study have been deposited in the European Nucleotide Archive (ENA) at EMBL-EBI under accession number PRJEB98675 (<https://www.ebi.ac.uk/ena/browser/view/PRJEB98675>).

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