Traditionally fermented foods still as a critical avenue impacting host gut antibiotic resistome

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Abstract

Disrupted gut microbiota as a critical risk factor for many noncommunicable diseases is largely driven by gut microbiota-impacting drugs, especially orally administered and biliary excreted antibiotics. Fermented food consumption has been encouraged to replenish disrupted gut microbiota; however, its overall impact on host gut health remains to be elucidated. This study examined traditionally fermented foods and the gut microbiota of consumers of fermented foods for antibiotic resistance. Dietary intervention with fermented foods was found to lead to a surge in the antibiotic resistome in the gut microbiota of most human subjects. The antibiotic resistome was further illustrated in traditionally fermented food samples, and antibiotic-resistant bacteria were isolated from approximately 90% of the retail kimchi and all artisan cheeses assessed in this pilot screening. The identified isolates included pathogens of importance in nosocomial infections such as *Klebsiella pneumoniae* and *Enterococcus*, as well as commensals and lactic acid bacteria, and some isolates exhibited extremely high minimum inhibitory concentration (MIC) against antibiotics of clinical significance. Exposing fermented food microbiota to representative antibiotics further led to a boost in the corresponding antibiotic and multidrug resistance gene pools and disturbed microbiota. The results revealed an underestimated public health risk associated with fermented food interventions, particularly in susceptible populations with gastrointestinal tract symptoms and compromised immune functions seeking gut microbiota rescue. These findings call for more comprehensive assessments of the benefits and potential safety challenges associated with traditionally fermented foods and strategic movements to mitigate unnecessary damage to the host gut microbiota and improve food safety.

Key words

Gut microbiota; traditionally fermented foods; dietary intervention; antibiotic resistance; resistome; opportunistic pathogens; kimchi; artisan cheese

Introduction

The rapid rise in antibiotic resistance (AR) negates the effective treatment of bacterial infections, shaking the foundation of modern medicine. AR bacteria in host gastrointestinal tract further indirectly contribute to gut microbiota dysbiosis resulting from even short-term antibiotic treatment.[1] Gut microbiota destruction has been recognized as a critical, shared risk factor for a growing list of noncommunicable "modern" diseases, ranging from malfunction of the host immune system, type-II diabetes, brain/neurological disorders, *Clostridium difficile* infections, to certain cardiovascular diseases and cancers.[2-10] Both AR and noncommunicable diseases are among the top global public health threats in the 21st century.[11]

For decades, the broad applications of antibiotics have been blamed for the rapid surge of AR and, more recently, for disrupted host gut microbiota and associated "modern" diseases. But despite limiting the uses of antibiotics has been the primary control strategy worldwide, even essential antibiotic applications for infection prevention and treatment still cause irreversible damages in host gut microbiota.[12,13] AR is a complicated issue with multiple risk factors.[14,15] Particularly, gut impacting antibiotics, i.e., the mainstream oral administration of antibiotics, and using drugs with primary biliary instead of renal excretion, rather than the application of antibiotics, have been the key and shared driver for the rapid surge of the antibiotic resistance gene (ARG) pool, massively disrupted gut microbiota, and the rise of pathogens in gut microbiota.[16,1] This paradigm-changing discovery is further supported by clinical observations on the trends of AR worldwide.[17,1] The finding is also applicable to non-antibiotic drugs with impact on gut microbiota.[18] According to CDC, over 200 million outpatient oral antibiotic prescriptions were given in the US annually.[19] Assuming even distribution, this is equivalent to

over 60% of the U.S. population alone affected every year, contributing to the global rising trends of the aforementioned diseases.

Given its impact on host health, various approaches to replenish damaged gut microbiota have been attempted. Fecal microbiota transplant (FMT) is gaining approval for treating *C. difficile* infections,[20,21] but the procedure was associated with acquired infections, including death by ESBL *E. coli*.[22] It further led to a surge in resistome in both human and gnotobiotic pig recipients after FMT procedures.[23] Autologous fecal microbiota transplantation (auto-FMT) facilitated the restoration of the gut microbiota and immune functions in patients.[24] Yet, re-assessing the gut microbiota profiles and resistome illustrated that auto-FMT using own fecal microbiota banked before disruptive drug therapy, with the intention of avoiding the introduction of infectious agents from other donor(s), still resulted in the rise of resistome and opportunistic pathogens in multiple recipients even without further antibiotic treatment (Wang et al., unpublished data. Communicated with authors of MKSCC with consensus on the above conclusion in January 2021).

Fermented foods have emerged in recent years as a popular alternative to repair disrupted gut microbiota.[25,26] Wastyk et al.[26] reported that consuming diets high in fermented foods increased diversity of the gut microbiota and improved immune function of the human recipients, while the same effect was not observed in subjects with diets high in plant fibers. Fermented vegetables, followed by kombucha and fermented dairy products (yogurt and kefir), were among the most consumed categories of fermented foods by this group of subjects (Supplemental Data STable 1).

However, fermented foods that are rich in viable microbes are susceptible to AR. Before 2007, fermented dairy foods had been the most significant foodborne avenue transmitting AR to

consumers.[27] A gram of retail cheese contained up to 10^8 copies of AR genes,[28] and the *Bifidobacterium* strain supplemented to yogurt products worldwide used to contain a tetracycline (Tet) resistance-encoding gene (personal communication, 2007 ASM General Meeting). Various bacteria isolated from fermented foods with mobile AR genes were able to cause acquired resistance in human commensals and pathogens via horizontal gene transfer mechanisms.[27,29,30] Although successful mitigation of the AR gene pool was quickly achieved in mainstream fermented dairy products by 2011, primarily by removal of problematic fermentation starter cultures and probiotic strains from the product lines,[31,15] other traditionally fermented products may still be prone to AR.[32-48] Unlike mainstream modern dairy fermentation, which uses pasteurized milk and commercial starter cultures carefully screened by major culture companies, traditionally fermented vegetables and artisan cheeses, for instance, still rely on microbiota associated with raw materials, environment, or "mother" cultures other than carefully screened commercial starters.[31-33,40,43,47,49]

The fermented food and beverage market is expected to grow by 5.6% in the next 10 years and will exceed US$ 989.2 billion by 2032 amid escalating demand for healthy and nutritious foods.[50] The COVID-19 pandemic has further facilitated the explosive growth of sales of fermented products such as sauerkraut and kimchi.[51] Given the booming market and increased consumption, re-assessing potential AR risks associated with traditionally fermented food products has become an urgent food safety and public health need, especially for susceptible populations with compromised digestive tract and immune systems. Therefore, this study investigated the incidence of antibiotic resistome in representative fermented foods and the impact of fermented food interventions on the human gut resistome.

Results

Impact of food intervention on fecal microbiota resistome. Ten-week dietary interventions with diets high in fermented foods or plant fibers led to 11 increased and 6 decreased, as well as 10 increased and 5 decreased gut antibiotic resistomes in human subjects, respectively, but the impact of the two types of diets was distinctive (Fig 1 & Fig 2). Fermented foods intervention led to significant changes in fecal antibiotic resistome of subjects with a p-value of 0.03, and the mean of resistome increased from 0.36 to 0.42 copies/16S rRNA gene (Fig 2A). Intervention with high plant fiber diets caused insignificant changes in the host gut resistome, with a p-value of 0.94. The mean resistome remained at 0.38 copies/16S rRNA gene in subjects before and after dietary intervention (Fig 2B). The summarized resistome data are illustrated in Supplemental Data STable 2. For details on the resistome data, see the Supplemental Data at the end (STables 7 and 8).

[Insert Fig 1 here]

[Insert Fig 2 here]

[Insert Table 1 here]

Antibiotic resistome of traditionally fermented foods. Results of the pilot survey on retail fermented foods supported the aforementioned impact on the host gut resistome. Multidrug resistance, bacitracin, and macrolide-lincosamide-streptogramin (MLS) genes were most abundant in pooled kimchi samples (K1-K4) recovered from brain heart infusion (BHI) plates (Fig 3A and Table 1), and they were also among the most abundant AR genes of all five individual kimchi microbiota (K7-K11) assessed directly, despite the exact abundance of the top AR genes varied among these samples (Table 1 & Fig 4A). Likewise, even though AR isolates were identified in eight kimchi samples (K5, K7-K13, Table 2), the overall abundance of the

resistome of the assessed samples also varied, ranging from 0.372 (K9) to 0.029 (K11) copies of AR genes/16S rRNA (Fig 4A).

[Insert Fig 3 about here]

[Insert Fig 4 about here]

[Insert Table 2 here]

Fig 4B further compares the resistome outcomes of kimchi sample K7 using total DNA extracted from kimchi microbiota directly (0.048 copies of AR genes/16S) and total DNA of kimchi microbiota recovered from BHI agar plates (2.909 copies of AR genes/16S). The results indicated that with specific medium and incubation conditions, even without antibiotic selective pressure, certain bacteria in the kimchi microbiota might have been selectively enriched, leading to a drastic difference in the detected antibiotic resistome. Likewise, the top genera of pooled kimchi microbiota recovered from BHI plates (Supplemental Data STable 3) were affected by cultivation conditions as well, as the illustrated resistome (Fig 4B).

Indeed, Table 3 illustrates that the top genera of each individual kimchi microbiota (K7 to K11) assessed using total DNA extracted directly were dominated by lactic acid bacteria, which are well-recognized for driving kimchi fermentation. The data further demonstrated that the antibiotic resistome and AR bacteria were still prevalent in these successfully fermented kimchi products.

[Insert Table 3 here]

The resistome results of pooled artisan cheese samples C1 to C4 (Fig 3B) and individual cheese samples C5 to C8 (Fig 4C), all using microbiota recovered from BHI agar plates, further

illustrated the prevalence of various AR genes in these products. Multidrug, MLS, and bacitracin were among the top AR genes of the pooled samples C1-C4, while multidrug, bacitracin, tetracycline, and MLS were the most abundant among individual samples C5 to C8 (Table 4). Notably, the abundance of the resistome of cheese microbiota recovered from BHI plates may also be affected by the medium and culture conditions, deviating from those of the original samples. Supplementary STable 5 further illustrated that *Staphylococcus* (including *Mammaliicoccus*, also known as *Staphylococcus*) was the dominant genus of individual artisan cheese microbiota recovered from BHI agar plates.

[Insert Table 4 here]

Representative AR bacteria from traditionally fermented foods. Various AR bacteria were isolated from traditionally fermented products. Under the very limited cultivation conditions applied in this study, AR bacteria were identified in eight out of nine kimchi and four out of four cheese samples assessed by culture recovery (Tables 2 and 5).

[Insert Table 5 here]

Identified AR isolates from kimchi products ranged from important human pathogens and opportunistic pathogens, such as *Klebsiella pneumoniae*, *Serratia marcescens* etc., which are recognized agents for nonsocomial infections,[52,53] to organisms still considered commensals, as well as lactic acid bacteria driving food fermentations. For instance, isolates of several species of *Latilactobacillus*, *Leuconostoc* and *Weissella* were among those highly resistant to the four antibiotics screened for, namely tetracycline (Tet), erythromycin (Ery), ampicillin (Amp), and vancomycin (Van). Some isolates exhibited multidrug resistance.

The AR isolates identified from cheese products were mostly *Staphylococcus* spp. resistant to tetracycline, and one isolate was resistant to Van. An isolate of *Enterococcus* sp. was highly resistant to all the four antibiotics assessed.

Antibiotic exposure further shaped the profiles of fermented food originated microbiota. As illustrated in Fig. 5 and Supplementary STable 5, the cheese microbiota harvested from BHI plates without antibiotics was dominated by *Mammaliicoccus* sp. (formerly *Staphylococcus*), *Staphyloccocus*, *Camobacterium*, *Psychrobacter* and *Glutamicibacter* (belonging to Micrococcaceae) for cheese sample C5. However, in the presence of Tet or Amp, the dominant bacteria were *Stenotrophomonas*, *Pseudomonas*, *Burkholderia*, *Alcanivorax* and *Streptococcus*. Likewise, while the dominant cultures for cheese C7 recovered on BHI were *Staphylococcus* and *Lactococcus*, the top-ranked bacteria of cheese-originated microbiota shifted to *Pseudomonas*, *Burkholderia*, *Staphylococcus*, *Streptococcus* and *Leuconostoc* on BHI-Amp and *Staphylococcus* on BHI-Tet. In the case of C7 and pooled sample C1-C4, the cheese starter culture *Lactococcus* was eliminated when the cheese microbiota was exposed to either of the two antibiotics.

[Insert Fig 5 about here]

Although antibiotic-containing agar plates were used to screen for AR bacteria, the above results also indicated how the food-originated microbiota might impact the gut microbiota when the hosts receive antibiotic treatment. *Stenotrophomonas*, *Pseudomonas*, *Burkholderia*, and *Staphylococcus* are opportunistic pathogens associated with antibiotic-resistant hospital-acquired infections, and most *Streptococcus* species are pathogenic.

Microbial profiles by metagenomics of dominant cheese bacteria recovered on BHI plates included *Staphylococcus*, *Enterococcus* and *Lactocaseibacillus* (formerly *Lactobacillus*) for

cheese sample C6, and *Enterococcus*, *Tetragenococcus*, *Staphylococcus*, *Lactococcus* and *Camobacterium* for sample C8 (Fig. 5 and Supplementary STable 5). In agreement with this, some of the mentioned antibiotic-resistant bacteria, such as multidrug-resistant *Enterococcus* sp. and *Staphylococcus* were also confirmed in these samples (Table 5).

Discussion and Conclusion

Although unexpected for many, the susceptibility of fermented foods to AR has been well-recognized at least in the food microbiology community. Since the first systematic demonstration of the problem in the early 2000s with a broad spectrum of AR isolates, including starter cultures, opportunistic pathogens, and commensals of mainstream fermented dairy products being identified and characterized,[27] AR bacteria have been isolated from various fermented foods worldwide.[54-56] For instance, *Pantoea agglomerans* (formerly *Enterobacter agglomerans* or *Erwinia herbicola*), an opportunistic pathogen that causes a wide range of opportunistic infections, especially in immunocompromised patients, was isolated from kimchi in South Korea. The genome of *P. agglomerans* isolate KM1 contains 13 genes conferring resistance to clinically important antibiotics, and the strain exhibited immunostimulatory properties *in vitro*, including the production of pro-inflammatory and anti-inflammatory cytokines in stimulated cells.[57]

This study, however, using a combination of approaches, for the first time systematically reveals the impact of dietary intervention by fermented foods on antibiotic resistome in human gut microbiome, backed by data on the prevalence and abundance of antibiotic resistome in popular fermented foods. The original human study on dietary intervention by Wastyk et al.[26] had a total of 36 healthy adult subjects who completed the study; half received fermented foods, and the other half received dietary fiber intervention. The sample size was small, providing the variables

for human subjects and activities. Among these, the fecal microbiome outcomes of 32 subjects with required data points, including 17 subjects with fermented food intervention and 15 subjects as control (high plant fiber diet), were assessed and reported for the impact of dietary intervention on the host gut resistome in this study.

Although the sample size of the human study was rather limited, the illustration of antibiotic resistome in kimchi and artisan cheese microbiome, along with the high prevalence of confirmed AR bacteria in approximately 90% of the kimchi and all artisan cheeses assessed in this pilot study, supports the conclusion that consuming these traditionally fermented products would result in an increase in the gut antibiotic resistome in consumers. Since only limited conditions were applied in cultivating AR bacteria, and resistance against only four antibiotics was assessed in this pilot study, the recovered isolates represented only a small percentage of the AR microbiota of the fermented foods. The results of this pilot study thus call for more extensive investigations on the food safety risks associated with traditionally fermented foods and for effective mitigation strategies to protect public health.

It is worth noting that even in subjects who consumed only yogurt and kombucha (Subject 8020) or yogurt, kombucha, and kefir (Subject 8014) (STables 2 and 6), the gut resistome of the subjects still increased by 11% and 17%, respectively, after the 10-week intervention. The data suggest that consuming these products at least did not reduce gut antibiotic resistance in the subjects.

The identification of AR bacteria from these products, ranging from human and plant pathogens to lactic acid bacteria that drive food fermentation, especially those exhibiting multidrug resistance and with extremely high MICs against antibiotics of clinical significance, is

particularly concerning. Multidrug-resistant *Klebsiella pneumoniae*, *Serratia marcescens*, as well as *Stenotrophomonas*, *Pseudomonas*, *Burkholderia*, *Staphylococcus* and *Streptococcus* spp. are also known to cause hospital-acquired infections, including some of the most troublesome cases in healthcare facilities. Thus, data from this study also provide an alternative interpretation of the route of dissemination and potential origins of infections in patients.

Balanced gut microbiota as well as effective mucosal, intestinal epithelial, and gut vascular barriers as part of an integral and functional gastrointestinal tract system are essential for host health.[58] Physical damage to the intestinal barriers has various health consequences. Intestinal tract problems such as inflammatory bowel diseases, enteric infections, or overgrowth by pathogens such as EHEC and Shiga toxin-producing *E. coli*, *Shigella*, *Salmonella*, *Campylobacter*, *C. difficile*, enteric viruses, and certain drug treatments such as the antibiotic clindamycin, and even alcohol consumption can cause acute or chronic intestinal barrier damages. Once the intestinal barrier collapses, intestinal microorganisms and their metabolites can enter the bloodstream, reach other organs, and cause further health damage. Once they enter the bloodstream, even commensals and probiotics can also be problematic. For instance, the popular probiotic strains *Bifidobacterium* and *L. rhamnosus* GG were found causative to bacteremia and sepsis in high-risk populations, with strain confirmation by metagenomic sequencing.[59-68] While bacteremia and sepsis by commercial probiotic strains absence of AR genes are still controllable by mainstream antibiotics such as ampicillin, introducing the broad spectrum of AR bacteria highly resistant to clinically important antibiotics associated with traditionally fermented foods presents an underestimated and potentially serious public health risk, especially to targeted populations suffering from various gut symptoms associated with "leaky gut" as well as compromised immune functions. Bacteremia and sepsis risk, antibiotic

therapy failures, and further gut microbiota destruction after antibiotic treatment may significantly increase in targeted susceptible consumer populations who intend to repair the damaged gut microbiome by enhancing fermented food consumption.

With the recipients of over 200 million oral antibiotic prescriptions added to the susceptible population suffering from broad gut microbiota destruction annually in the US alone,[19] the impacted patient population worldwide is astonishing. While various gut microbiota-replenishing approaches, including fermented food intervention, have been practiced desperately, they all have introduced additional public health risks to patients. The results from this study thus further call for strategic efforts to tackle the key and shared driver of the antibiotic resistome surge and gut microbiota disruption in humans and animals by mitigating the applications of gut-impacting antibiotics (oral administration and biliary excretion) and offering alternatives to significantly minimize unnecessary damages to the gut microbiome for productive outcomes.

Methods and Materials

Host gut resistome assessment. In the original study by Wastyk et al.[26], 39 healthy adult participants were recruited, with 18 randomly assigned to the dietary intervention group by fermented foods, and the remaining 21 were assigned to the plant fiber intervention group. Three participants in the fiber intervention group dropped out of the study; thus, both groups had 18 participants who completed the entire study (25 female,11 male, with an average age of 52 ± 11 years). Fecal shotgun metagenomics data of these participants were collected at four checkpoints, (Week -2, Week 0, Week 8, and Week 10) throughout the study and deposited in the NCBI BioProject database (ID number: PRJNA743361). After initial screening for data availability,

three participants in the fiber group and one participant in the fermented food group were excluded from our study because of missing Week 10 endpoint shotgun sequencing data in the database provided by the original research team. Raw sequencing data were retrieved from the Sequence Read Archive (. sra) format, and converted to. fastq using the NCBI SRA Toolkit (version 3.0.0) and further processed on a high-performance supercomputer at the Ohio Supercomputer Center.

For quality control, the FASTP tool (version 0.22.0) was used (parameters: -q 20 -u 20 -n 2 -l 80) to clean and trim the raw sequences before further annotation and analysis, as described by Chen et al.[69]. The cleaned sequences were then processed using the ARGs-OAP pipeline (Online Analysis Pipeline for Anti-biotic Resistance Genes, version 2.5).[70] The resistome analysis criteria were set as hit length of 50%, e-value of 1e-07 and identity of 80%. The generated data were further analyzed and plotted using Tidyverse packages, including ggplot2 (version 3.3.6), dplyr (version 1.0.10), ggstatsplot package (version 0.10.0), and car package (Companion to Applied Regression, version 3.1-1) on R version 4.2.1.[71-75]

Data transformation and analysis. The sum of the total ARG was calculated by adding up the relative abundances of all types of ARG annotated by ARGs-OAP. The percentage of total resistome change for each subject was calculated using the following equation: (∑resistome reads after dietary intervention- ∑resistome reads before dietary intervention)/ ∑resistome reads before dietary intervention × 100%. The resistome data from Week -2 and Week 0 were averaged as the baseline to ensure that all subjects had baseline data before dietary intervention and to reduce natural variation in gut microbiota. Normality and variance of the data sets were verified using the Shapiro-Wilk normality test and Levene's test. Violin-boxplots with Student's t-test p-values ($\alpha<0.05$) were generated using the ggstatsplot package.[74] Other plots were

constructed using the ggplot2 package.[72] Summary statistics such as mean, median, standard deviation, and significance of dietary intervention were calculated using the dplyr package.[75]

Source of fermented food samples. Representative kimchi samples of different brands were purchased from seven retail stores, one chain restaurant, and two local Japanese and Korean restaurants in Columbus, OH, between 2021 and 2022. Artisan cheese products were purchased from a national grocery chain store in Columbus, OH, with four purchased in 2021 and four in 2022. Sample designation and brief descriptions are illustrated in Supplementary Data STable 1.

Recovery and assessment of viable bacteria from fermented food samples. Five grams of each food sample were stomached in 45 ml 0.1% peptone water using a Seward stomacher 80 (Seward, UK). The juice was assessed for total bacteria and AR bacteria using Brain Heart Infusion (BHI), Luria-Bertani (LB), or De Man, Rogosa, and Sharpe (MRS) agar plates with cycloheximide or nystatin as a mold inhibitor, with or without the corresponding antibiotic (32 µg/mL Amp; 32 or 64 µg/mL Tet; 2 µg/mL Ery for BHI or MRS). The plates were incubated at 30 °C under aerobic or anaerobic conditions. Single colonies recovered from antibiotic-containing agar plates were picked based on their morphology and identified by Sanger sequencing of 16S rRNA gene PCR amplicons.[27] The MIC of recovered AR isolates against four commonly used antibiotics was determined by a microdilution procedure,[76] but using the corresponding recovery media instead.

Antibiotic resistome of representative fermented food items. Twenty-five grams of food sample was mixed with 25 ml 0.1% peptone water and stomached as described above. Large particles of food debris were removed using sterile sieves. The liquid phase was centrifuged at 1711 relative centrifugal force using Multifuge X1R (Thermo Scientific, USA, MA) for 5 min. The pellet was washed once with 0.1% peptone water and subjected to total DNA extraction using a QIAamp PowerFecal Pro DNA Kit (Qiagen, Hilden, Germany) following the manufacturer's instructions.

Alternatively, the culture-recovered microbiota was scraped from agar plates with 3mL of 0.1% peptone water and subjected to DNA extraction using the QIAamp kit.

The DNA samples were subjected to shotgun metagenomic sequencing (2X155bp) with an average depth of 16.7 to 20 million paired-end reads for each sample, using an Illumina NextSeq2000 Sequencing System. The raw sequences were cleaned (parameters: -q 20 -u 40 -n 2 -l 80) and analyzed using the same metagenomic tools and parameters as described above to obtain the relative abundance of ARGs normalized to the number of 16S rRNA genes. The sequencing data were further assessed for microbiota profiles through the Kraken2 pipeline with a standard database and minimum-base-quality 20, as described previously.[77] the food microbiota profile of Kimchi #7 was assessed by 16S rRNA sequencing using an Illumina MiSeq system.

All local metagenomic data analyses were conducted using a high-performance supercomputer at the Ohio Supercomputer Center.

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Part of the data was presented at the 2022 ASM Microbe Satellite/Sino-Micro annual meeting (Washington, DC) and USDA multistate NC1206 project meeting on antibiotic resistance (Ames, IA).

Data availability statement

The metagenomic sequencing data were deposited in the NCBI BioProject database (ID number: PRJNA944373).

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Tables

Table 1. Most abundant AR genes in kimchi samples[1].

Sample #	Rank				
	1	2	3	4	5
K1-4 (BHI, pooled)	Multidrug 0.3189*	Bacitracin 0.0901	Unclassified 0.0780	MLS** 0.0684	Fosfomycin 0.0465
K1-4 (BHI-Tet, pooled)	Multidrug 0.2556	Tetracycline 0.1059	Unclassified 0.0777	Bacitracin 0.0485	MLS 0.0422
K1-4 (BHI-Amp, pooled)	Multidrug 0.3235	Bacitracin 0.1795	β-lactam 0.0680	MLS 0.0557	Fosfomycin 0.0498
K7 (BHI)	Multidrug 1.7085	Unclassified 0.3728	Bacitracin 0.1609	MLS 0.1278	Fosmidomycin 0.1216
K7 (BHI)	unclassified 0.0248	Multidrug 0.0118	Bacitracin 0.0050	MLS 0.0023	fosmidomycin 0.0012
K8 (BHI)	Multidrug 0.0681	Unclassified 0.0469	Bacitracin 0.0136	MLS 0.0047	Tetracycline 0.0006
K9 (BHI)	MLS 0.2557	Multidrug 0.0577	Unclassified 0.0390	Bacitracin 0.0191	Vancomycin 0.0001
K10 (BHI)	Bacitracin 0.0880	Unclassified 0.0669	Multidrug 0.0182	MLS 0.0009	Fosfomycin 0.0005
K11 (BHI)	Unclassified 0.0191	Bacitracin 0.0063	Multidrug 0.0026	Tetracycline 0.0002	MLS* 0.0002

*Numerical readings of AR: total ARG copies per 16S rRNA gene.

**MLS: Macrolide-Lincosamide-Streptogramin.

Table 2. Summary of identified AR colonies from kimchi products purchased in 2022*.

Isolate	Identity	Food source	Recovered from	Resistance with MIC* (µg/mL)
K5-1	*Latilactobacillus (Lactobacillus) sakei*	Kimchi 5	MRS-tet	Tet (>64)
K5-2	*Bacillus (licheniformis/paralicheniformis)*	Kimchi 5	BHI-ery	Amp (>64) Ery (>16)
K5-3	*Weissella sp.*	Kimchi 5	MRS-ery	Amp (>256) Ery (>16) Tet (>128)
K7-5	*Serratia quinivorans*	Kimchi 7	BHI/BHI-amp	Amp (>64) Van (>1024)
K7-6	*Klebsiella pneumoniae*	Kimchi 7	BHI/BHI-amp	Amp (>256) Van (>1024)
K7-8	*Enterobacter sp.*	Kimchi 7	BHI/BHI-amp	Amp (>256) Van (>1024)
K7-9	*Rahnella aquatilis*	Kimchi 7	BHI/BHI-amp	Amp (>256) Ery (>100) Van(>1024)
K8-6	*Bacillus licheniformis*	Kimchi 8	BHI-ery	Amp (>64) Ery (>16)
K9-1	*Latilactobacillus (Lactobacillus) curvatus*	Kimchi 9	MRS-tet	Tet (>64)
K9-2	*Bacillus (licheniformis/paralicheniformis)*	Kimchi 9	BHI-ery	Amp (>64) Ery (>16)
K9-3	*Latilactobacillus (Lactobacillus) sakei*	Kimchi 9	MRS-ery	Ery (>16)
K10-1	*Levilactobacillus (Lactobacillus) brevis*	Kimchi 10	BHI-tet	Tet (>64) Van (>512)
K10-2	*Levilactobacillus (Lactobacillus) brevis*	Kimchi 10	BHI-tet	Tet (>64) Van (>512)
K11-3	*Rahnella aquatilis*	Kimchi 11	MRS-amp	Amp (>256)
K12-1	*Leuconostoc citreum*	Kimchi 12	BHI-tet	Tet (>128)
K12-6	*Latilactobacillus (Lactobacillus) sakei*	Kimchi 12	BHI-tet	Tet (>32)
K13-1	*Serratia marcescens*	Kimchi 13	BHI-tet	Amp (>256) Ery (>200) Tet (>128) Van (>1024)
K13-2	*Oceanobacillis jeddahense*	Kimchi 13		Tet (>32) Ery (>16)

*Tested MIC for representative antibiotics, including vancomycin (Van), ampicillin (Amp), erythromycin (Ery), and tetracycline (Tet).

Table 3. Top 5 genera classified of microbiota directly of 5 kimchi samples purchased in 2022.

Sample	Top genera	Percentage
Kimchi7	*Leuconostoc*	94.87%
	Other	3.17%
	Rahnella	0.85%
	Weissella	0.48%
	Levilactobacillus	0.36%
	Xanthomonas	0.27%
Kimchi8	*Latilactobacillus*	55.98%
	Leuconostoc	28.90%
	Lactiplantibacillus	7.49%
	Other	5.46%
	Lactococcus	1.59%
	Levilactobacillus	0.58%
Kimchi9	*Latilactobacillus*	81.60%
	Leuconostoc	11.79%
	Other	3.45%
	Weissella	2.02%
	Lactiplantibacillus	0.59%
	Levilactobacillus	0.55%
Kimchi10	*Lactiplantibacillus*	54.60%
	Leuconostoc	32.89%
	Levilactobacillus	9.80%
	Other	1.86%
	Weissella	0.55%
	Latilactobacillus	0.30%
Kimchi11	*Weissella*	58.85%
	Leuconostoc	39.19%
	Other	1.41%
	Latilactobacillus	0.23%
	Lactiplantibacillus	0.19%
	Stenotrophomonas	0.13%

Table 4. Most abundant AR genes in BHI plates recovered microbiota of artisan cheese samples[1].

Sample #	Rank				
	1	2	3	4	5
C1-4 (BHI, pooled)	Multidrug 0.4704*	MLS** 0.0783	Bacitracin 0.0353	Fosfomycin 0.0265	Tetracycline 0.0105
C1-4 (BHI-Tet, pooled)	Tetracycline 0.5377	Multidrug 0.2359	Unclassified 0.0876	Bacitracin 0.0744	MLS 0.0295
C1-4 (BHI-Amp, pooled)	Multidrug 0.0308	Tetracycline 0.0093	MLS 0.0084	Bacitracin 0.0053	Unclassified 0.0033
C5 (BHI)	Multidrug 0.1343	Fosfomycin 0.1123	Unclassified 0.0709	β-lactam 0.0617	MLS 0.0555
C5 (BHI-Tet)	Multidrug 0.0033	β-lactam 0.0031	Tetracycline 0.0014	Bacitracin 0.0012	unclassified 0.0012
C5 (BHI-Amp)	Multidrug 0.0040	β-lactam 0.0038	unclassified 0.0015	bacitracin 0.0014	MLS 0.0013
C6 (BHI)	Multidrug 0.6202	Tetracycline 0.4674	Bacitracin 0.2046	Unclassified 0.1750	MLS 0.0694
C7 (BHI)	Multidrug 0.3538	Tetracycline 0.2740	Unclassified 0.1442	Bacitracin 0.1308	MLS 0.0557
C7 (BHI-Tet)	Multidrug 0.2558	Tetracycline 0.2215	Unclassified 0.1183	Bacitracin 0.1176	Quinolone 0.0167
C7 (BHI-Amp)	Multidrug 0.0394	Bacitracin 0.0085	Tetracycline 0.0078	Unclassified 0.0075	β-lactam 0.0035
C8 (BHI)	Multidrug 0.2605	Unclassified 0.0737	Vancomycin 0.0671	Bacitracin 0.0472	MLS 0.0345

*Numerical readings of total ARG copies per 16S rRNA gene.

**MLS: Macrolide-Lincosamide-Streptogramin.

Table 5. Summary of identified AR colonies from artisan cheese products purchased in 2022*.

Isolate	Identity	Food source	Recovered from	Resistance with MIC* (µg/mL)
C5-3	*Staphylococcus equorum*	Cheese 5	BHI-ery	Ery (>16)
C5-9	*Mammaliicoccus vitulinus*	Cheese 5	BHI-ery	Ery (>16)
C6-2	*Staphylococcus xylosus*	Cheese 6	BHI-tet	Tet (>64)
C6-4	*Staphylococcus saprophyticus*	Cheese 6	BHI-tet	Tet (>64)
C6-10	*Staphylococcus xylosus*	Cheese 6	BHI-tet	Tet (>64) Van (>64)
C6-11	*Enterococcus faecalis*	Cheese 6	MRS-ery	Amp (>128) Tet (>128) Ery (>16) Van (>64)
C7-1	*Staphylococcus xylosus*	Cheese 7	BHI-tet	Tet (>32)
C7-5	*Staphylococcus saprophyticus*	Cheese 7	BHI-tet	Tet (>64)
C8-11	*Staphylococcus equorum*	Cheese 8	BHI-ery	Ery (>16) Van (>16)

*Tested MIC for representative antibiotics, including vancomycin, ampicillin, erythromycin, and tetracycline.

Figures

Fig 1. Impact of 10-week food intervention on the change of antibiotic resistome of fecal microbiota of human subjects in percentage comparing to the baseline. Green: by foods rich in fermented products; pink: by foods rich in plant fibers.

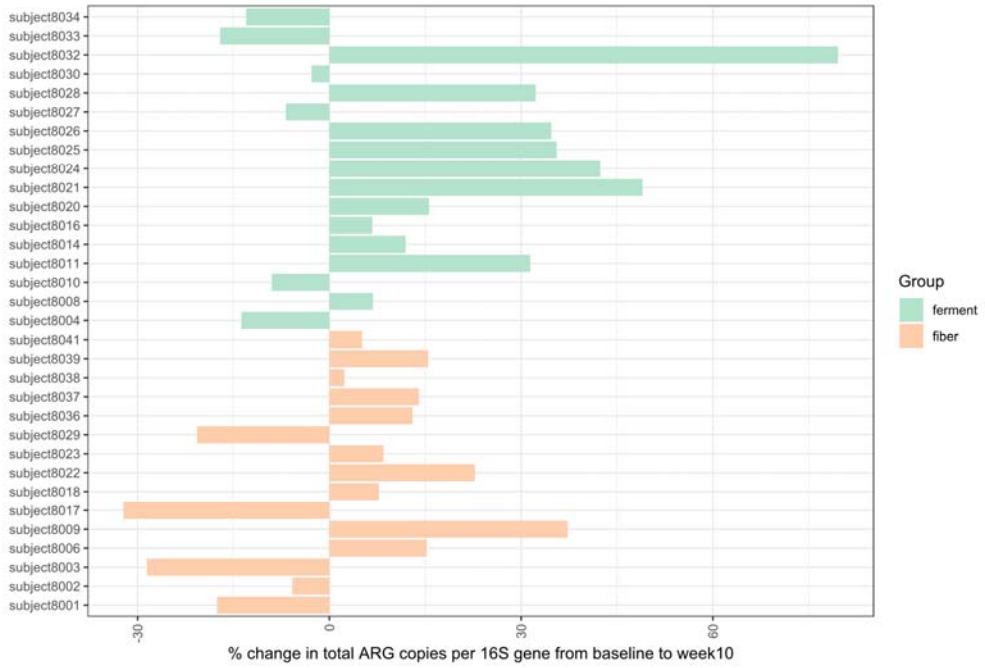
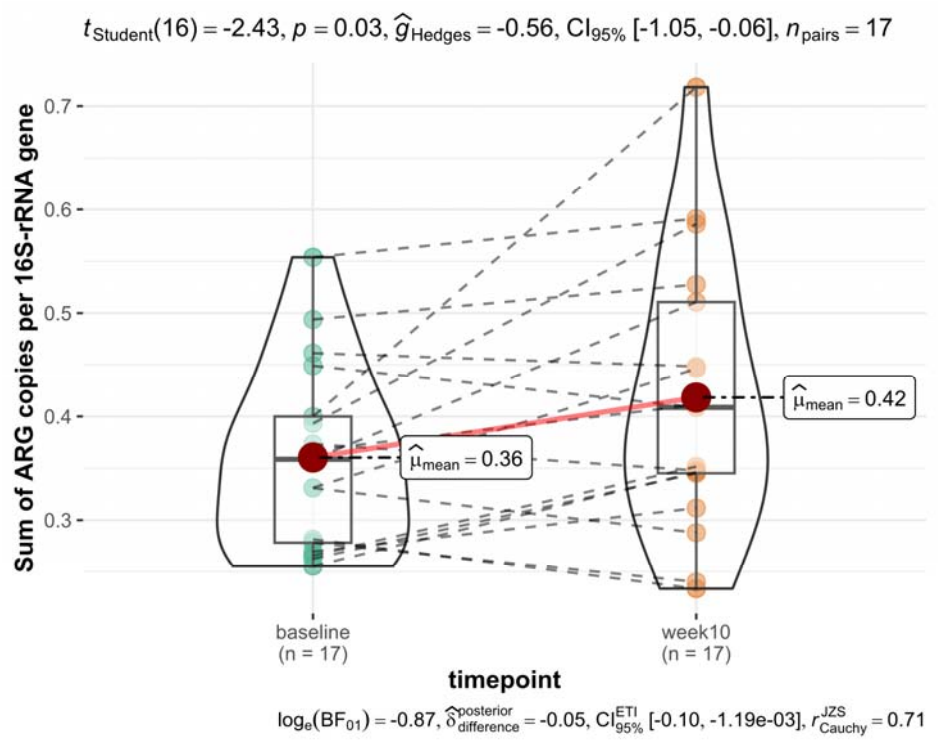


Fig 2. Statistical significance analysis of the impact of 10-week food intervention on the change of antibiotic resistome of fecal microbiota of human subjects in percentage comparing to the baseline by diets rich in A) fermented foods; B) plant fibers.

A)



B)

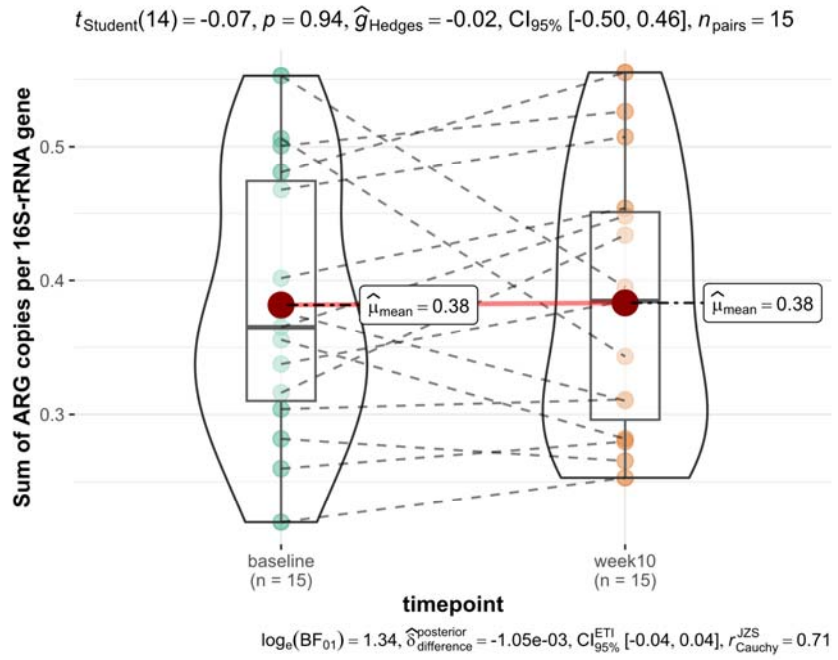
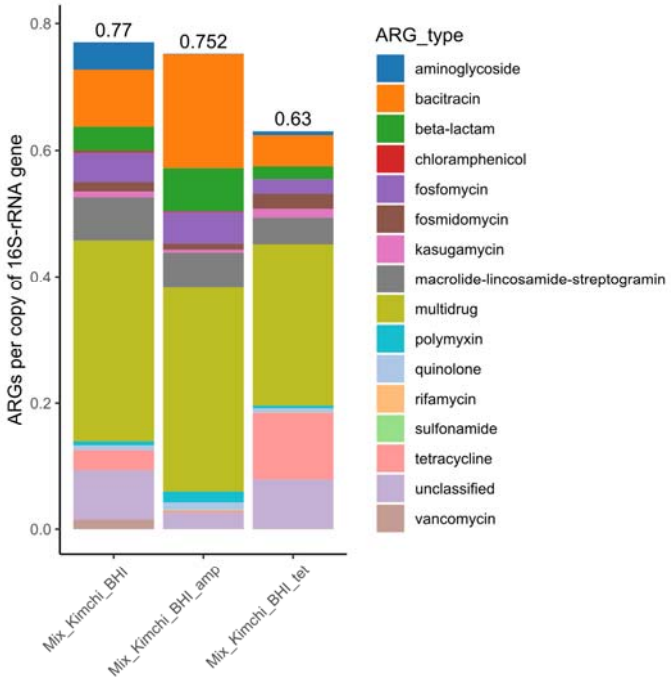


Fig 3. Antibiotic resistome of pooled traditionally fermented food microbiota recovered from agar plates. A) 4 kimchi samples (K1-K4) purchased in 2021. B) 4 artisan cheeses (C1-C4) purchased in 2021.

A).



B).

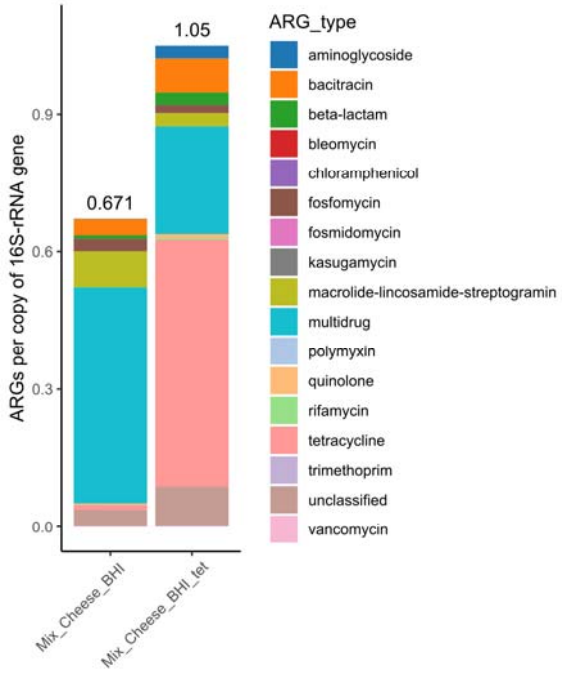
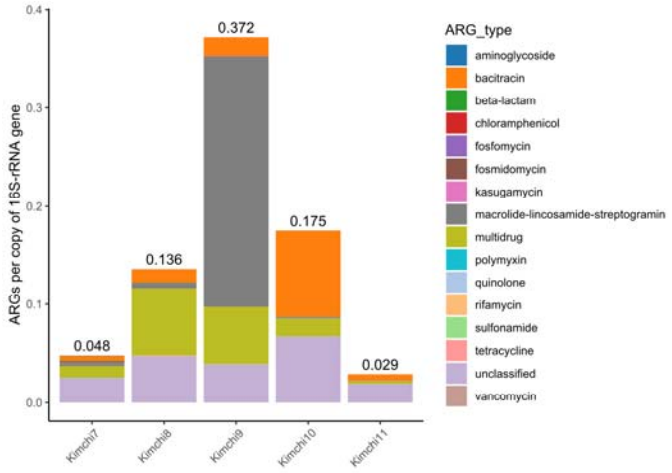
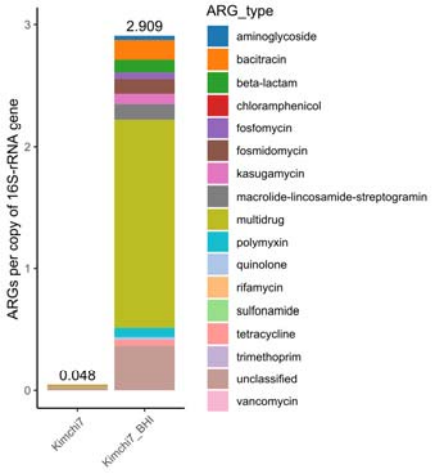


Fig 4. Resistome assessment of individual fermented foods purchased in 2022. A) resistome of direct kimchi microbiota of samples; B) resistome of direct kimchi microbiota (left bar) and BHI recovered microbiota (right bar) of kimchi sample K7; C). resistome of BHI recovered cheese microbiota.

A).



B).



C).

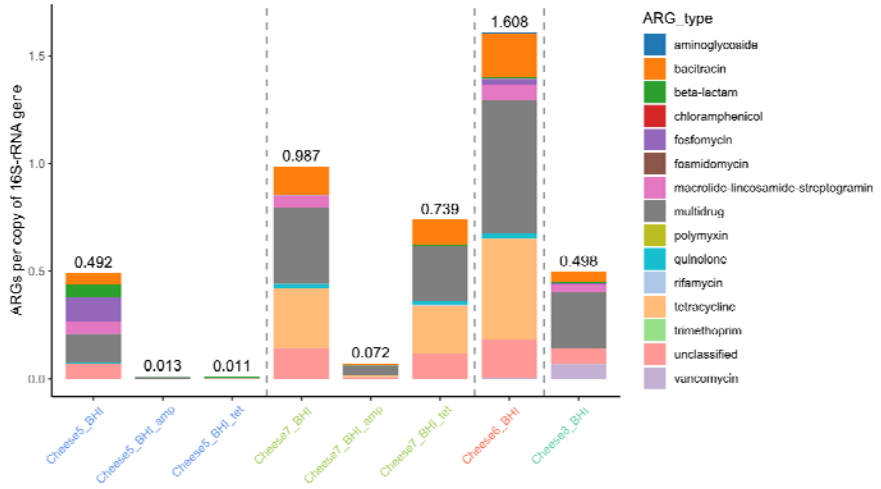
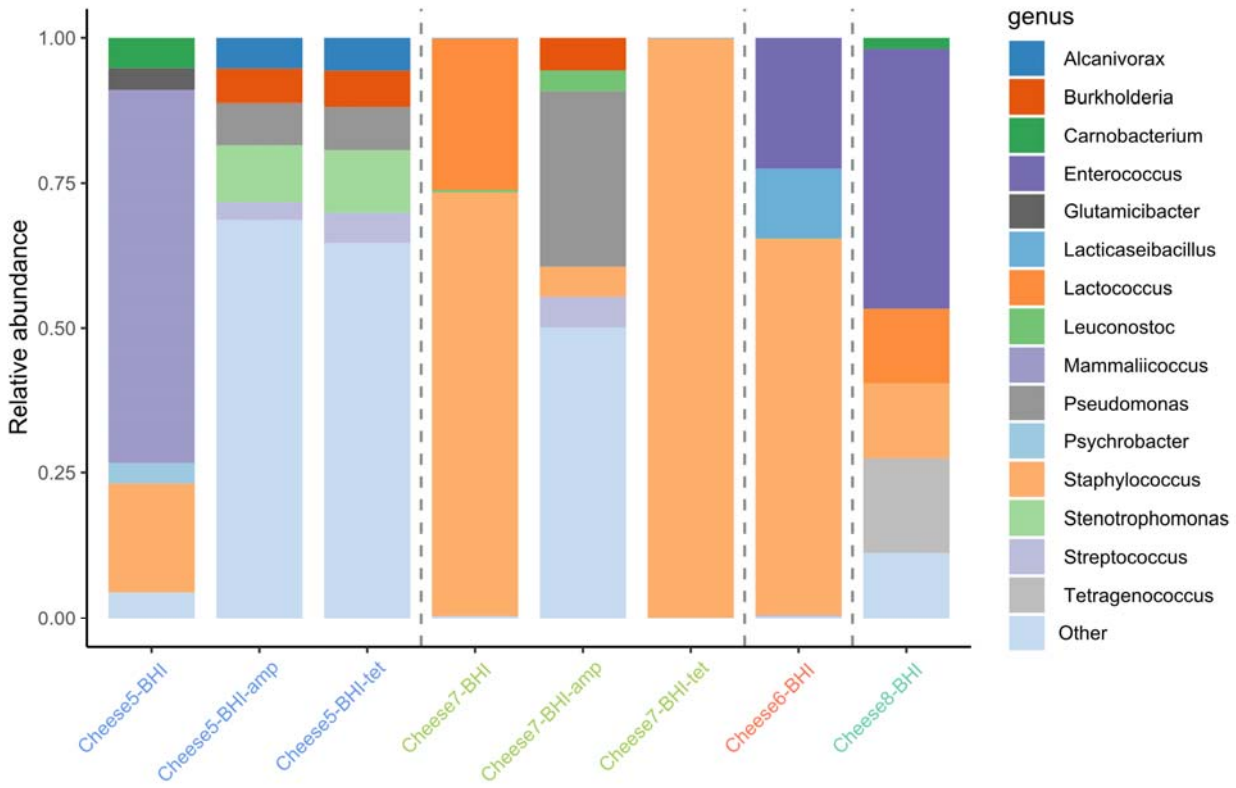


Fig 5. Detected cheese microbiota recovered from BHI agar plates with and without antibiotics.



Supplemental tables

STable 1. Fermented food samples assessed in this study.

Sample #	Description	Year of Purchase	Plate recovered microbiota extraction shotgun	Direct sample extraction shotgun	AR isolate screening	AR isolate illustrated
Kimchi 1 (K1)	From Korean market A	2021	+ (mixed)			
Kimchi 2 (K2)	From Korean restaurant A	2021	+ (mixed)			
Kimchi 3 (K3)	From Japanese restaurant A	2021	+ (mixed)			
Kimchi 4 (K4)	From Korean restaurant B	2021	+ (mixed)			
Kimchi 5 (K5)	From Korean restaurant B	2022			+	+
Kimchi 6 (K6)	Bottled brand A	2022			+	
Kimchi 7 (K7)	From a Korean chain restaurant C	2022	+	+	+	+
Kimchi 8 (K8)	Bottled brand B	2022		+	+	+
Kimchi 9 (K9)	Bottled brand C	2022		+	+	+
Kimchi 10 (K10)	Bagged brand D	2022		+	+	+
Kimchi 11 (K11)	From Japanese market B	2022		+	+	+
Kimchi 12 (K12)	Bottled brand E	2022			+	+
Kimchi 13 (K13)	From Korean Market A	2022			+	+
Cheese 1 (C1)	X(i)* Blue, pasteurized milk (Germany)	2021	+ (mixed)			
Cheese 2 (C2)	X(ii)*, raw cow's milk (Aged > 60 days) (France)	2021	+ (mixed)			
Cheese 3 (C3)	X(iii)* Blue, pasteurized cow and sheep milk (France)	2021	+ (mixed)			
Cheese 4 (C4)	X(iv)* Blue, organic pasteurized milk	2021	+ (mixed)			

	(Unknown)					
Cheese 5 (C5)	C(i)*, pasteurized buffalo milk (Italy)	2022	+		+	+
Cheese 6 (C6)	C(ii)*, raw cow's milk (Aged > 60 days) (United States)	2022	+		+	+
Cheese 7 (C7)	C(iii)*, raw cow's milk (Aged > 60 days) (United States)	2022	+		+	+
Cheese 8 (C8)	C(iv)*, pasteurized part-skim cow's milk (Switzerland)	2022	+		+	+

*Cheese products were coded to protect the identities of individual producers.

STable 2. Gut antibiotic resistome of subjects with dietary intervention[1].

	Total ARG copies/16S gene				
Subject ID	"Week-02"	Week0	Week08	Week10	Intervention by
8004	0.272186	0.284192	0.300493	0.239926	Fermented foods
8008	0.479228	0.508536	0.404436	0.527567	Fermented foods
8010	0.438165	0.460143	0.377093	0.408678	Fermented foods
8011	0.283978	0.241384		0.345206	Fermented foods
8014		0.367376	0.420609	0.411247	Fermented foods
8016	0.608693	0.498886	0.611231	0.591077	Fermented foods
8020	0.251731	0.287428		0.311607	Fermented foods
8021	0.357738	0.428821	3.77E-01	0.585925	Fermented foods
8024	0.345131	0.372126	0.364187	0.510634	Fermented foods
8025	0.188706	0.322113	0.308409	0.346233	Fermented foods
8026		0.33126	0.515311	0.446267	Fermented foods
8027	0.414694	0.332016	0.455848	0.347911	Fermented foods
8028	0.265774			0.351583	Fermented foods
8030	0.407724	0.514995	0.598983	0.448453	Fermented foods
8032	0.383879	0.416004	0.468486	0.718217	Fermented foods
8033	0.290082	0.272963	0.236222	0.23334	Fermented foods
8034	0.297562	0.364338	0.363455	0.287874	Fermented foods
8001		0.376419	0.316027	0.310167	High plant fibers
8002	0.274209	0.289648	0.264493	0.265522	High plant fibers
8003	0.624062	0.481308	0.447535	0.394988	High plant fibers
8006	0.219233	0.219967	0.233956	0.253023	High plant fibers
8009	0.182759	0.449417	0.415087	0.433871	High plant fibers
8017	0.765387	0.246743		0.342993	High plant fibers
8018	0.259782		0.241797	0.279882	High plant fibers
8022	0.364975		0.534551	0.44805	High plant fibers
8023		0.467738	0.385584	0.507301	High plant fibers
8029	0.397652	0.313326	0.326775	0.281955	High plant fibers
8036	0.381629	0.421726		0.45393	High plant fibers
8037	0.354639	0.320164	0.283361	0.384654	High plant fibers
8038	0.338513	0.269464	0.420975	0.311128	High plant fibers
8039	0.409468	0.552285	0.551152	0.555141	High plant fibers
8041	0.499627	0.501698	0.510558	0.526228	High plant fibers

[1]Numerical readings in total ARG copies per 16S gene.

STable 3. Top 5 genera classified of microbiota recovered from BHI agar plates of pooled 4 kimchi samples purchased in 2021.

Sample	Top genera	Percentage
Mix_Kimchi_BHI	*Bacillus*	58.29%
	Serratia	13.42%
	Pseudomonas	12.11%
	Leuconostoc	9.92%
	Other	4.72%
	Lelliottia	1.55%
Mix_Kimchi_BHI_amp	*Pseudomonas*	46.63%
	Bacillus	41.71%
	Brucella	5.92%
	Enterobacter	2.71%
	Other	1.70%
	Rahnella	1.34%
Mix_Kimchi_BHI_tet	*Latilactobacillus*	34.33%
	Bacillus	33.42%
	Serratia	18.88%
	Stenotrophomonas	5.65%
	Lactococcus	4.14%
	Other	3.57%

STable 4: Top 5 genera classified of microbiota recovered from BHI agar plates of pooled 4 cheese samples purchased in 2021.

Sample	Top genera	Percentage
Mix_Cheese_BHI	*Lactococcus*	47.32%
	Lacticaseibacillus	25.26%
	Staphylococcus	23.19%
	Other	2.04%
	Enterococcus	1.09%
	Streptococcus	1.09%
Mix_Cheese_BHI_tet	*Staphylococcus*	52.84%
	Bacillus	31.53%
	Streptococcus	12.24%
	Microbacterium	1.92%
	Other	1.29%
	Bacteroides	0.17%

STable 5: Top 5 genera classified of BHI-recovered microbiota of individual cheese samples purchased in 2022.

Sample	Top genera	Percentage
Cheese5-BHI	*Mammaliicoccus*	64.41%
	Staphylococcus	18.62%
	Carnobacterium	5.26%
	Other	4.49%
	Glutamicibacter	3.73%
	Psychrobacter	3.51%
Cheese5-BHI-amp	Other	68.49%
	Stenotrophomonas	9.89%
	Pseudomonas	7.20%
	Burkholderia	5.91%
	Alcanivorax	5.31%
	Streptococcus	3.19%
Cheese5-BHI-tet	Other	64.56%
	Stenotrophomonas	11.03%
	Pseudomonas	7.46%
	Burkholderia	6.17%
	Alcanivorax	5.66%
	Streptococcus	5.14%
Cheese6-BHI	*Staphylococcus*	64.68%
	Enterococcus	22.47%
	Lacticaseibacillus	12.22%
	Other	0.29%
	Streptococcus	0.28%
	Leuconostoc	0.07%
Cheese7-BHI	*Staphylococcus*	73.00%
	Lactococcus	26.05%
	Leuconostoc	0.40%
	Other	0.32%
	Lacticaseibacillus	0.13%
	Streptococcus	0.11%
Cheese7-BHI-amp	Other	50.11%
	Pseudomonas	30.32%
	Burkholderia	5.63%
	Staphylococcus	5.18%
	Streptococcus	5.16%
	Leuconostoc	3.60%
Cheese7-BHI-tet	*Staphylococcus*	99.71%
	Other	0.10%
	Enterococcus	0.09%
	Leuconostoc	0.05%

	Mammaliicoccus	0.03%
	Lactococcus	0.02%
Cheese8-BHI	*Enterococcus*	44.89%
	Tetragenococcus	16.18%
	Staphylococcus	13.08%
	Lactococcus	12.68%
	Other	11.26%
	Carnobacterium	1.91%