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Are fermented foods an overlooked reservoir of antimicrobial resistance?

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Despite their many cultural, culinary, and health benefits, fermented foods may amplify and disseminate antimicrobial resistance in our food supply. This review summarizes our current understanding of the diversity, distribution, and potential risks of antimicrobial resistance in fermented foods and beverages. Most studies have focused on antibiotic resistance genes (ARGs) in lactic acid bacteria and coagulase-negative *Staphylococcus* species. Resistance to tetracyclines, penicillins, chloramphenicol, and macrolides is frequently reported. Several studies have demonstrated that ARGs have the potential to be transferred from fermentation microbes to pathogens. Most research has used culture-based or metagenomic surveys or ARGs at the point of production, and few studies have traced the fate of ARGs when ferments are consumed. Cases of humans being directly harmed by resistant microbes in ferments have not been reported, but these foods provide a farm-to-gut pipeline for current and future antimicrobial resistance in our food supply.

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Introduction

Fermented foods contain bacteria and fungi that transform raw materials into more flavorful foods with a longer shelf-life [1,2]. Unpasteurized and uncooked ferments have the potential to deliver viable microbes to the human microbiome [3,4]. There is growing evidence that these microbial cultures can have positive impacts on nutritional qualities of fermented foods and may even have direct probiotic effects on human health [5].

In addition to their many potential benefits, fermented foods may also harbor microbes with undesirable traits that could pose long-term risks for human microbiomes. Microbes in the food supply that are resistant to antimicrobials have received increased scrutiny over the past several decades as human and animal pathogens have become resistant to clinically important antibiotics [6]. Food may be an important reservoir of both resistant pathogens as well as antibiotic resistance genes (ARGs) in nonpathogenic microbes that could eventually be transferred to pathogens. Most research on ARGs in food has focused on pathogenic microbes given the direct threat of these microbes to human health [7]. But recent studies have begun to catalog the resistome of nonpathogenic microbes in different parts of the global food system [8]. By understanding these reservoirs of resistance in nonpathogenic microbes, we may be able to better understand and manage the emergence of antibiotic-resistant pathogens.

Fermented foods may pose unique antimicrobial resistance risks compared to other commonly consumed foods. Most resistant microbes in ferments are nonpathogenic, meaning that a direct threat to human health is unlikely. But even the presence of genetically labile ARGs in beneficial fermentation microbes could be problematic. Many fermented foods are consumed raw, and the microbial cells in ferments have opportunities to interact with various components of the human microbiome [3–5]. The densities of microbial cells in many ferments is high per gram of food consumed [4], meaning that ARGs in fermented food microbiomes could be at a higher density compared to other foods that are consumed raw (vegetables, fruits, etc.).

When considering the types of antimicrobial resistance across fermented foods and the potential risks they pose, it is important to first consider how microbes can be resistant to antibiotics. One way is through intrinsic resistance, where an entire species or many species in a genus lack the target of an antibiotic or have other mechanisms for being resistant. For example, many fermented food microbes have intrinsic resistance to key antibiotics, including the widely known resistance of some members of the Lactobacillaceae to vancomycin, some aminoglycosides, ciprofloxacin, and trimethoprim [9]. Because of this intrinsic resistance, most studies of fermented foods that look for antibiotic resistance will

find it. But that does not mean that these foods necessarily pose a safety risk [10] as this form of resistance is rarely horizontally transferred. In contrast, acquired resistance can be due to chromosomal mutations in existing genes in the genome or due to the acquisition of new genetic material through horizontal gene transfer (HGT) [11,12]. It is the latter type of mobile resistance that is the main concern in the production and consumption of fermented foods because it has the potential to move across species boundaries, from beneficial microbes in ferments to human commensals or pathogens.

There have been hundreds of reports of ARGs in fermented foods over the past several decades [13]. This wealth of data would suggest that antimicrobial resistance in fermented foods is a widespread problem. But these individual reports from specific bacteria and specific foods do not put antimicrobial resistance in fermented foods into a broader food system context. Additionally, past reviews of antimicrobial resistance in fermented foods have often focused on specific organisms or specific foods [14–18], so patterns across different types of foods, production systems, and geographies have not been identified. The goal of this overview is to highlight emerging views of the diversity, distribution, and risks of antimicrobial resistance across different types of ferments.

How is antimicrobial resistance disseminated across fermented food systems?

Because of the complexities and interdependencies of our modern food systems, it is challenging to carefully track the origin and dissemination of antimicrobial resistance for individual products or regions [19]. Even the spread of deadly and highly resistant pathogens is challenging to trace from farm environments to consumers [20]. Most studies of ARGs in fermented foods have focused on isolation and characterization of resistant microbes in specific fermented foods at the point of production [13]. How upstream activities affect the abundance of ARGs in ferments has not been directly studied.

Studies of how ARGs spread within other food systems provide a framework for fermented foods (Figure 1) [6]. One of the largest microbial inputs in fermentation production is the use of starter cultures. These defined microbial strains are often added to ferments to help control the consistency of product flavor and aesthetics. Before they are widely disseminated from culture companies, it is expected that starter cultures are screened for safety parameters, including the presence of resistance genes, and some regulations exist in Europe regarding antibiotic resistance in food and feed cultures [21]. But many studies have demonstrated that starter cultures can contain ARGs for antibiotics that are still used in humans [16]. Continued scrutiny of potentially transferable resistance genes in starter cultures is necessary to ensure that this easy-to-control source of microbes in ferments is not a vector of resistance.

Another way that microbes with mobile ARGs are introduced into fermented food production systems is through the raw materials used for fermentation. In meat and dairy ferments such as cheeses and fermented meats, animal-associated microbes can ultimately become part of the fermented food microbiome [22,23]. These animals may have received antibiotics in the past to treat an infection or may have acquired ARGs from exposure to other animals [24]. Unlike animal production systems, plant production systems are not typically exposed to high concentrations of antibiotics. For ARGs to be abundant on the raw materials used in grain, vegetable, or fruit fermentations, those materials would need to be exposed to microbial sources containing resistant microbes. Several ARG reservoirs in crop systems include soils, water, and manure [25,26].

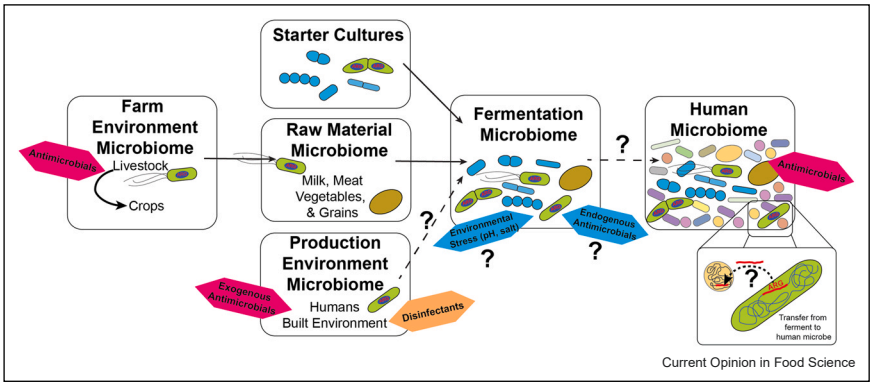
The human and built environment microbes in fermented food production systems could also harbor ARGs that could be transferred to fermented foods. Many studies have documented the diverse resistome of human and built environment microbiomes [11,27,28]. But whether microbes can move from these sources into ferments has not been clearly demonstrated. Several studies have highlighted considerable overlap between the humans or buildings where ferments are made and the ferments produced in those facilities [29–32], but directionality of potential transfer between the products, people, and environment is not clear.

Once microbes with ARGs are present in ferments, there is potential for their ARGs to spread to other fermentation microbes via HGT [33]. Studies in food model systems have demonstrated that mobile genetic elements containing resistance genes can be transferred from one bacterial species to another [34]. In some cases, transfer in the ferment was higher than transfer using standard agar plating techniques [34]. Most of these studies have been done with artificially inoculated ferments in highly controlled conditions. Future studies using emerging techniques to study HGT of resistance [35] will reveal how often ARGs are transferred within ferments under more realistic conditions.

Has transfer of antimicrobial resistance from fermented food microbes to human-associated microbes been demonstrated?

For antimicrobial resistant microbes from fermented foods to pose a direct risk to human health, ARGs need

Figure 1



A conceptual overview of how microbes with ARGs may move from sources to fermented foods, and ultimately to the human gut microbiome. Green cells indicate microbes with ARGs. Dotted lines note connections across foods systems that have not been established, but may exist. Question marks highlight major unknowns in the diversity and dissemination of resistance in fermented foods.

to be transferred from ferment-associated microbes to human-associated microbes. These can either be human commensals that serve as an ARG reservoir, or the transfer could happen directly from ferment microbes to pathogens. The transfer of ARGs from fermentation-associated microbes to human-associated microbes has been clearly demonstrated *in vitro* using direct contact of microbes on agar plates [36–38]. Data from *in vivo* studies with murine models are much more limited, but also suggest a potential for ARG transfer from ferments to the human microbiome [39,40]. For example, a recent study assessed whether a *Staphylococcus equorum* isolate from a fermented seafood product could transfer resistance to an isolate of *Staphylococcus saprophyticus* [40–43], a cause of urinary tract infections. When the two strains were fed to germ-free mice, a plasmid conferring resistance to lincomycin was transferred from *S. equorum* to *S. saprophyticus* in the absence of lincomycin selection.

While these *in vitro* and *in vivo* experiments can certainly demonstrate the potential for resistance transfer, they are done under highly artificial conditions that microbes do not experience in natural systems. Microbes are often grown in rich media prior to potential transfer events, even though nutrients are much more limiting in food, gut, and other environments. Donor (fermentation-associated) and recipient (human-associated) strains are also typically grown in a 1:1 ratio with no other microbes present. In more realistic conditions, the ratios would be highly variable, and hundreds or thousands of other species could be present. These studies are critical for demonstrating the potential for and mechanisms of resistance transfer, but are lacking in realism and do not allow for accurate risk assessment. More realistic studies that track the horizontal transfer of resistance from fermentation derived microbes to the human microbiome are needed [44].

What types of antibiotic resistance genes have been detected in fermented foods?

Using both culture-based and metagenomic approaches, a range of ARGs have been identified in different bacteria of widely consumed ferments (Table 1). Most studies have focused on ARGs in lactic acid bacteria (LAB) [17,18,45]. When only considering evidence for the presence of active and acquired ARGs in LAB, one common pattern across many papers is that resistance to tetracyclines, penicillins, chloramphenicol, and macrolides such as erythromycin is very widespread (Table 1). Perhaps not surprisingly, these are some of the most commonly used antibiotics for growth promotion and infection control in livestock, and ARGs that resist these drugs are widespread in livestock microbiomes [46]. Most resistant LAB have been identified in some types of cheeses, fermented meats, and spontaneously fermented vegetables [14,17,47]. Considerable focus has been on LAB in the Lactobacillaceae as well as some *Enterococcus* species that can be opportunistic pathogens [48].

Other widespread fermentation bacteria where ARGs have been frequently reported are the coagulase-negative *Staphylococcus* (CNS) species. CNS species are abundant in fermented animal products, including some meats, cheeses, and fermented fish products [42]. Concern about ARGs in CNS comes from their potential to colonize humans (*S. saprophyticus, S. xylosus*) and because they may have the potential to transfer resistance to pathogenic *Staphylococcus* species. As with LAB, the most common types of ARGs reported in CNS confer

Table 1

Distribution of antimicrobial risks and ARGs in widely[a] produced and consumed fermented foods and beverages.

Food	Relative Risk of Antibiotic Resistance	Potential Microbes of Concern	Common[b] Types of Potentially Mobile ARGs	Key Papers
Fermented meat (e.g. sausage, salami)	**High (for some meat products[c]).** Animal-associated microbes in some products, including raw meat products	CNS; LAB	**CNS:** Penicillins and tetracycline **LAB**: Tetracycline, chloramphenicol, erythromycin, penicillins	[15,17,58]
Cheese	**High (for some cheeses[c]).** Animal-associated microbes in some products, especially raw-milk cheeses	CNS; GNB; LAB	**CNS:** Tetracycline, chloramphenicol, erythromycin **GNB:** Inconclusive based on current data **LAB**: Tetracycline, chloramphenicol, erythromycin	[45,47,52,59]
Fermented vegetables (e.g. sauerkraut, kimchi)	**Medium.** Frequent potentially transferable resistance in LAB.	LAB	**LAB**: Tetracycline, chloramphenicol, erythromycin, clindamycin	[14]
Kefir	**Low.** Infrequent transferable resistance in LAB reported in literature.		Limited evidence for acquired ARGs in the literature	[60,61]
Yogurt	**Low.** Most yogurt is inoculated with defined starter cultures that should be free of ARGs		Limited evidence for acquired ARGs in the literature	
Sourdough breads	**Low**. Baked, so microbes are not viable when consumed. Bakers may be exposed to microbes with ARGs through contact with starters		Limited evidence for acquired ARGs in the literature	
Vinegars and kombucha	**Low.** Limited ARGs reported in AAB. Kombucha yeasts may be resistant, but not studied		Limited evidence for acquired ARGs in the literature	[53,54]
Wine, beer, and other alcoholic beverages	**Low.** Most microbes not viable (or have been filtered out) before consumption		Limited evidence for acquired ARGs in the literature	

[a] This table only includes broad classes of fermented foods and beverages that are made and produced around the world. We recognize that a huge diversity of culturally and economically significant ferments are not included in this table. Our goal is to provide a broad overview, not an exhaustive review. Please see [13] for additional details.
[b] This is not an exhaustive list and only includes acquired ARGs that have been clearly documented using phenotypic and genotypic assays across several studies from different geographic areas. Exact resistances observed will depend on where and how the product is made.
[c] Some meat and cheese products may have a high abundance of starter cultures or may be heat treated, decreasing the risk of exposure to antibiotic resistant microbes.

resistance to tetracyclines, penicillins, chloramphenicol, and macrolides (Table 1).

Beyond LAB and CNS, considerably less attention has been paid to the resistance of other groups of microbes that are found in ferments. Gram-negative bacteria (GNB) are not found in many ferments because they are often eliminated by low pH, salt, ethanol, and other stressors that may be present in ferments. But some surface-ripened cheeses can have a high abundance of Gram-negative bacterial genera such as *Serratia*, *Pseudomonas*, *Hafnia*, and *Proteus* that are related to human pathogens [49–51]. A survey of antibiotic resistance in GNB from French cheeses found a range of resistance to common antibiotics [52]. A metagenomic study of cheeses also identified plasmids of putative GNB that were multidrug resistant [53].

Acetic acid bacteria (AAB) are widespread in many fermentations, including vinegar and kombucha. However, little attention has been paid to the antibiotic resistance of fermentation AAB. A metagenomic study of a broad range of ferments found few resistance genes in several kombucha samples, suggesting a limited potential for AAB to harbor ARGs [53]. However, a recent study using isolates of *Acetobacter* and *Komagaetaeibacter* species did identify potential resistance to chloramphenicol, ciprofloxacin, erythromycin, and trimethoprim in both vinegar and kombucha [54]. Whether AAB have intrinsic or acquired resistance to these antibiotics has not yet been determined.

Almost all research on antimicrobial resistance in ferments has focused on bacteria. But many fermented foods contain fungi that are consumed as living cells. Could antifungal resistance in fermented foods pose a risk to human health? In contrast to bacteria, resistance to antifungal drugs in fungi generally does not move via horizontal transfer [55], so concerns about resistance genes being passed from fermented food microbes to human pathogens and commensals are limited.

Despite the decreased risk of resistance transfer in fermentation fungi, some yeasts found in ferments may be potential opportunistic pathogens of humans and could be resistant to clinically relevant antifungals. A comparative genomic study of the yeast *Pichia kudriavzevii* demonstrated that clinically relevant and fermented food isolates of *Pichia kudriavzevii* are genetically similar, suggesting that foods and other environmental sources may be a source of this opportunistic pathogen [56]. Several isolates from food were resistant to multiple antifungal drugs, suggesting that production or consumption of fermented foods could lead to exposure to resistant strains of this pathogen. A study of milk kefir yeasts also demonstrated fluconazole resistance in a yeast commonly found in ferments (*Saccharomyces unisporus*), but this fungus is not known to be an opportunistic pathogen of humans [57].

Open questions and future research needs in fermented food antimicrobial resistance

The studies noted above are not intended to be exhaustive, but clearly demonstrate that fermented foods are a potential reservoir of ARGs. When consumers ingest some types of fermented foods, there is a high likelihood they are consuming viable microbes that possess labile ARGs. There is a chance that these ARGs could be transferred to their human microbiome, but this has not been directly demonstrated. While fermented foods do not appear to harbor ARGs that confer resistance to most of the critical 'last resort' antibiotics, there is clear data demonstrating that fermented food production systems are potential vectors of a variety of different ARGs. We have a lot of work to do before we fully understand the basic biology, risk assessment, and management of ARGs in fermented foods. Below are a few research areas that could help begin to fill some of these gaps.

How do antibiotic resistance genes move from farms to ferments and ultimately to the gut? What are critical control points that might limit the dissemination of antibiotic resistance genes in fermentation production systems?

Because most previous research has only focused on a single point in a food production system, it is hard to understand how resistant microbes and their ARGs move from raw materials into fermentation systems and then how they may interact with the human gut. It is challenging and costly to study fermentation microbiology with a systems-level perspective where all components of the system (farms, raw materials, production facilities, workers, aging environments, etc.) can be carefully monitored over time for ARGs.

Can abiotic and biotic conditions in ferments select for resistant microbes?

It is surprising to see such a high frequency of strains with acquired resistance genes in ferments when there is no apparent selection to maintain the resistance (no antibiotics). These microbes may have other traits that determine their success and allow their ARGs to persist without selection. But there may also be aspects of the abiotic or biotic environment of ferments that could select for resistant microbes. For example, in some fermented meats and cheeses, there is a high abundance of filamentous fungi that can secrete penicillin or other antimicrobial compounds [62,63]. These fungi could select for resistant bacteria present in low amounts in the raw materials used for fermentation. Additionally, adaptation to abiotic environments can select for antibiotic resistance in the absence of antibiotic exposure [64]. Future studies using experimental evolution of fermentation microbes will help better define how

fermentation environments may unintentionally drive the evolution of antimicrobial resistance.

Do antibiotic resistance genes in fermented foods pose a greater risk compared to other foods?

Many foods are consumed raw, including many fruits and vegetables. Consumers may also be exposed to ARGs in raw meats during preparation for cooking. Do fermented foods pose a greater risk for exposure compared to these other foods? Is there a unique diversity or frequency of ARGs in ferments compared to other foods? Unfortunately, few studies have systematically measured the distribution of ARGs in fermented versus other food categories, so we lack a clear answer to this question. A metagenomic survey suggested that the frequency of ARGs in cheese is not greater than the ARGs already present in the human microbiome [65]. A survey of foods in Switzerland suggested that the frequency of resistant microbes in cheese was lower than raw meat, but higher than fresh fruits and vegetables [66]. To really understand the risks of ARGs, more comprehensive and systematic surveys across many food systems are needed.

Does increased consumption of fermented foods lead to a higher incidence of antibiotic resistance genes in the human microbiome?

The connections between ferment and human microbiomes are poorly characterized, making it hard to clearly pinpoint risks of consuming ferments in terms of ARG exposure. Several experimental and observational studies of humans who have consumed fermented foods have illustrated potential shifts in the human microbiome associated with live microbes from fermented foods passing through the human digestive tract [3,5,67,68]. But none of these studies have directly measured how fermented food consumption impacts the diversity and frequency of ARGs in the human microbiome.

How does the frequency and abundance of fermented food antibiotic resistance genes vary in similar ferments made in different parts of the world or across farming practices?

Accessibility to antibiotics, regulations that control antibiotic use, and food system structures vary widely across geographic regions and between organic and conventional farming systems. To better tease apart how specific food production practices affect the abundance of ARGs in fermented foods, it would be useful to compare the types of ARGs in fermented foods across different geographic regions or across food production systems that use very different farming practices. It will also be important to study how the frequency of ARGs changes as new bans on antibiotic use go into effect.

Are there ways to manage the risk of antibiotic resistance genes in fermentation systems?

Even without a clear systems-level view of how ARGs move within fermentation production systems, there are several case studies that illustrate potential management strategies that can reduce consumer exposures to ARGs in fermentation systems [69]. These case studies are sparse and are often product-specific, but they may provide general insights that can be broadly applied to control antimicrobial resistance in ferments. For example, if it aligns with the fermentation process parameters, pasteurization of raw food materials can help eliminate environmental microbes harboring ARGs. Additionally, the use of starter cultures that are known to not harbor ARGs should be a widely implementable mitigation strategy. Many ferment producers already use these approaches, but they may need to be applied and fine-tuned in higher-risk ferments discussed above. More generally, reductions in the use of antibiotics across our global food systems should ultimately help reduce entry and spread of ARGs in fermented food production systems.

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