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Are spontaneously fermented plant-based foods potential sources of transferable antibiotic resistance genes?

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Abstract
Spontaneously fermented plant-based foods are highly valued for their unique organoleptic characteristics and presumed health benefits. However, because natural (spontaneous) fermentation does not involve the employment of starter cultures, the safety of spontaneously fermented plant-based products is not easily controlled. This review examines the presence of antibiotic resistance determinants in lactic acid bacteria (LAB) from plant-based, naturally fermented foods. Phenotypic resistance to clinically relevant antibiotics has been widely observed in LAB from a variety of food types. The larger proportion of the resistance traits reported in this review can be considered intrinsic to LAB. However, some of the identified traits, especially resistance genes to tetracycline and erythromycin, have the potential for horizontal spread. Further molecular characterization is required to determine the genetic basis of detected resistance traits that have only been assessed using phenotypic methods. The implementation of novel safety measures in the production of spontaneously fermented plant-based foods should be considered in the future.

KEYWORDS
antimicrobial resistance, fermented foods, food safety, lactic acid bacteria, plant-based foods, spontaneous fermentation

1 | INTRODUCTION

Fermented foods can be defined as foods or beverages produced through controlled microbial growth and the conversion of food components through enzymatic action (Marco et al., 2017). The process of fermentation involves the production of various metabolites that can inhibit the growth and survival of unfavorable microorganisms (Ross et al., 2002). Hence, throughout history, it has played a crucial role in the preservation of otherwise quickly perishable foods. Although fundamental, extended shelf life is not the only rationale for fermenting foodstuffs. Fermentation provides other benefits such as desirable organoleptic characteristics (e.g., taste, aroma, texture) or increased functionality of raw materials (e.g., use of barley for beer making). Recently, increased attention has also been given to the nutritional and health value of fermented products. The suggested mechanisms by which fermented foods might provide health benefits include changes in the nutritional content of the raw product, synthesis of bioactive peptides, modification of gut microbiota, and immune system stimulation (Dimidi et al., 2019; Marco et al., 2021).

Lactic acid fermentation depends on the transformations induced by lactic acid bacteria (LAB), that is, the conversion of carbohydrates into organic acids. The decrease in pH following these reactions inhibits
the growth of most spoilage and pathogenic microorganisms (Bautista-Gallego et al., 2020). Additionally, some LAB produce bacteriocins—antimicrobial peptides that may further inhibit undesirable microbial growth. The key genera classified as LAB include Lactobacillus, Lactococcus, Enterococcus, Streptococcus, Leuconostoc, Weissella, and Pediococcus (Ross et al., 2002).

The raw materials used for lactic acid fermentation encompass a diverse range of foods such as cereals, fruits, vegetables, milk, meat, and fish. Some of the widely known examples of fermented foods include cheese, yoghurt, kimchi, tempeh, sourdough, and kombucha (Tamang & Kailasapathy, 2010).

Foods can be fermented either by spontaneous (natural) fermentation or with the use of starter cultures (Hutkins, 2006). Natural fermentation relies on the autochthonous (indigenous) microorganisms present in the substrates. Being the oldest way of carrying out fermentation, this method has been utilized in the manufacture of many traditional products. Although some meat and dairy products are spontaneously fermented, spontaneous fermentation is more prevalent in vegetable, fruit, and cereal produce. Several requirements must be met for an uncontrolled fermentation to succeed, for example, suitable environment and presence of desirable microorganisms (Holzapfel, 2014). However, even when adequate conditions are provided, the fermentation outcomes may not meet the safety and quality expectations.

The unpredictability of spontaneously fermented produce has resulted in the widespread use of starter cultures, especially on an industrial scale. Fermentation with starter cultures involves inoculation of raw materials with a defined microbial culture so that it dominates the microflora and creates expected changes (Capozzi et al., 2017). The use of the starter shortens the fermentation period, simultaneously allowing for better control of sensory, safety, and quality aspects (Dolci et al., 2020). Bacterial starters may be used for a variety of foods but are more commonly utilized in the mass production of fermented meat and dairy products such as yoghurts, cheeses, or sausages. To be authorized as a starter culture, bacterial strains must meet certain safety standards, which in the European Union are regulated by the European Food Safety Authority (EFSA). One of the criteria requires that microorganisms intended for food use must lack transferrable antibiotic resistance (AR) genes (EFSA, 2012; Laulund et al., 2017).

AR poses one of the greatest global public health challenges and has been extensively researched in pathogenic species. However, limited studies have investigated AR in commensal bacteria (MacLean & San Millan, 2019). Bacteria in food might act as AR reservoirs, a phenomenon related most likely to antibiotic misuse in the food chain (WHO, 2011). Not all resistance traits can be transferred, however. Intrinsic (inherent) resistance, described as resistance typical to all strains of given species or genus, is unlikely to be spread. Conversely, acquired resistance (unless emanating from genomic mutation) presents a risk of being horizontally transferred. Such resistance involves the acquisition of genes located on conjugative or mobilizable plasmids and transposons from the bacterial donor (von Wintersdorff et al., 2016).

Fermented foods, an abundant source of LAB and other microbes, might constitute a vehicle for antimicrobial-resistant bacteria, particularly if the products are consumed without prior heat treatment. In that case, bacteria in possession of AR are likely to be carried over to the gut (Hummel et al., 2007). As a consequence, ingested resistant bacteria add to the pool of AR genes present in the large intestine and therefore increase the likelihood of transfer to pathogenic bacteria. Spontaneously fermented foods present a particular concern because fermentative bacteria are naturally present in the matrix and thus, they are not subject to safety assurance (Capozzi et al., 2017; Laulund et al., 2017). Due to the direct association of antibiotic use with livestock (and consequently fermented animal products), less attention has been given to investigate AR in plant-based fermented products. Many plant-based products are not fermented with a starter culture. Therefore, this review aims to examine the potential of LAB from plant-based spontaneously fermented foods as sources of transferable AR genes (Figure 1).

2 | OVERVIEW OF SPONTANEOUSLY FERMENTED PLANT-BASED FOODS

A wide variety of plant raw material, including fruits, vegetables, and cereals, are spontaneously fermented worldwide. Traditionally, this has been used in their preservation (Ogrodowczyk & Drabinska, 2021; Tamang et al., 2020). Some of the more well-known products include kimchi, sauerkraut, table olives, and pickled vegetables (lacto-fermented pickles). Examples of spontaneously fermented plant-based foods, including the main microorganisms involved, are summarized in Table 1. Recent reviews on the subject have been published by Ashaolu and Reale (2020), Torres et al. (2020), and Voidsarou et al. (2021).

There is increasing interest in plant-based fermented foods, as they are sources of potentially probiotic microorganisms, and present nondairy alternatives for the delivery of probiotics (Ogrodowczyk & Drabinska, 2021). Furthermore, such foods produced through spontaneous fermentation by LAB can be consumed without further heat treatment, possibly because of the low pH, making them a source of live microorganisms. However, it is not uncommon for fermented vegetables to be heat processed in order to further increase their shelf life (Rezac et al., 2018).

3 | POTENTIAL OF SPONTANEOUSLY FERMENTED PLANT-BASED FOODS AS SOURCES OF TRANSFERABLE AR GENES

The multiplication of bacteria that occurs during fermentation means that there would be an increase in the abundance of AR genes contained in the fermented foods. If any of the AR genes are mobile, then this increased abundance would raise the chances of horizontal gene transfer (HGT) occurring between the LAB and other bacteria in the gastrointestinal tract (Toth et al., 2020).

A selection of studies published between 2011 and 2020 are summarized in Table 2, showing AR traits in LAB from spontaneously fermented plant-based products. The foods from which LAB were
isolated include kimchi (Dey et al., 2019; Lee et al., 2011; Lee et al., 2016; Son et al., 2017; Won et al., 2020), sauerkraut (Touret et al., 2018; Yu et al., 2013), table olives (Argyri et al., 2013; Benitez-Cabello et al., 2019; Botta et al., 2014; Casado Munoz et al., 2014; Guantario et al., 2018), boza (Gotcheva et al., 2018; Valledor et al., 2020), tempoyak (Chuah et al., 2016; Khalil et al., 2018), fermented juices and nectar (Nawaz et al., 2011; Somashekarah et al., 2019; Xu et al., 2018), various fermented fruits and vegetables (Damodharan et al., 2015; Mahasneh et al., 2015; Michalak et al., 2018; Nawaz et al., 2011; Tang et al., 2018; Zielinska et al., 2015), and various “pickles” (Huang et al.,

**FIGURE 1** Schematic representation of the aim of this review

**TABLE 1** Examples of spontaneously fermented plant-based foods

<table>
<thead>
<tr>
<th>Fermented food type</th>
<th>Region</th>
<th>Main raw materials</th>
<th>Key lactic acid bacteria involved</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kimchi</td>
<td>Korea</td>
<td>Chinese cabbage (Napa cabbage), other vegetables, spices</td>
<td>Lactobacillus spp., Leuconostoc spp., Weissella spp., Lactococcus spp.</td>
<td>Patra et al. (2016)</td>
</tr>
<tr>
<td>Table olives</td>
<td>Italy, Spain, Greece</td>
<td>Olives</td>
<td>Lb. pentosus, Lb. plantarum</td>
<td>Perpetuini et al. (2020)</td>
</tr>
<tr>
<td>Lacto-fermented cucumber pickles</td>
<td>Europe, United States, Canada</td>
<td>Cucumbers</td>
<td>Lb. plantarum, Lb. brevis, Ln. mesenteroides, Pedicoccus acidilactici</td>
<td>Tamang et al. (2016) Behera et al. (2020)</td>
</tr>
<tr>
<td>Boza</td>
<td>Turkey, Bulgaria</td>
<td>Cereals (wheat, maize, millet, rice)</td>
<td>Lactobacillus spp., Pedicoccus spp., Lactococcus spp., Leuconostoc spp.</td>
<td>Tamang et al. (2016)</td>
</tr>
<tr>
<td>Tempoyak</td>
<td>Malaysia, Indonesia</td>
<td>Durian fruit</td>
<td>Lb. brevis, Lb. fermentum, Ln. mesenteroides, Lb. mali</td>
<td>Swain et al. (2014) Behera et al. (2020)</td>
</tr>
<tr>
<td>Investigated product</td>
<td>Investigated microorganisms</td>
<td>Phenotypic resistance traits detected</td>
<td>References</td>
<td></td>
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<tr>
<td>Boza (fermented cereal beverage)</td>
<td><em>Lactobacillus plantarum</em> &lt;br&gt;<em>Enterococcus faecium</em></td>
<td>VAN AMP TET CHL STR GEN RFP CIP ERY</td>
<td>Gotcheva et al. (2018) &lt;br&gt;Vallender et al. (2020)</td>
<td></td>
</tr>
<tr>
<td>Makdoos (fermented aubergines)</td>
<td><em>Lb. plantarum</em>&lt;br&gt;<em>Lb. pentosus</em>&lt;br&gt;<em>Lb. brevis</em>&lt;br&gt;<em>Lb. salivarius</em></td>
<td>AMP CIP ERY KAN GEN STR TET TMP</td>
<td>Mahasneh et al. (2015)</td>
<td></td>
</tr>
<tr>
<td>Pickled cabbage</td>
<td><em>Lb. rhamnosus</em>&lt;br&gt;<em>L. plantarum</em>&lt;br&gt;<em>L. johnsonii</em></td>
<td>STR VAN TET GEN</td>
<td>Zielinska et al. (2015)</td>
<td></td>
</tr>
<tr>
<td>Pickled cucumber</td>
<td><em>Lb. plantarum</em>&lt;br&gt;<em>Lb. casei</em>&lt;br&gt;<em>Lb. brevis</em></td>
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<td></td>
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<tr>
<td>Fermented cornelian cherry</td>
<td><em>Lb. plantarum</em>&lt;br&gt;<em>Lb. casei</em></td>
<td>CIP STR AMK GENATM FOX SXT</td>
<td>Tang et al. (2018)</td>
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</tr>
<tr>
<td>Fermented curly kale</td>
<td><em>Weissella helenica</em>&lt;br&gt;<em>W. cibaria</em>&lt;br&gt;<em>Leuconostoc mesenteroides</em>&lt;br&gt;<em>Lb. curvatus</em>&lt;br&gt;<em>Lb. plantarum</em>&lt;br&gt;<em>Lb. brevis</em>&lt;br&gt;<em>Lb. paraparallarum</em>&lt;br&gt;<em>Pediococcus pentosaceus</em>&lt;br&gt;<em>P. acidilactici</em>&lt;br&gt;<em>Lactococcus lactis</em></td>
<td>KAN STR NA TMPBC PIR PEN</td>
<td>Michalak et al. (2018)</td>
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</tr>
<tr>
<td>Fermented fruit and vegetable juices</td>
<td><em>W. cibaria</em>&lt;br&gt;<em>Ln. mesenteroides</em>&lt;br&gt;<em>Cc. lactis</em>&lt;br&gt;<em>P. pentosaceus</em>&lt;br&gt;<em>E. hirae</em>&lt;br&gt;<em>Lc. garvieae</em>&lt;br&gt;<em>W. confusa</em>&lt;br&gt;<em>Lb. plantarum</em>&lt;br&gt;<em>Streptococcus thermophilus</em></td>
<td>AMP PEN NOR STR AMK ERY LVX</td>
<td>Xu et al. (2018)</td>
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<tr>
<td>Fermented radish</td>
<td><em>Lb. plantarum</em>&lt;br&gt;<em>Lb. pentosus</em>&lt;br&gt;<em>Lb. fermentum</em></td>
<td>KAN GEN VAN STR</td>
<td>Damodharan et al. (2015)</td>
<td></td>
</tr>
<tr>
<td>Fermented vegetable</td>
<td><em>Lb. animalis</em>&lt;br&gt;<em>Lb. brevis</em>&lt;br&gt;<em>Lb. parabuchneri</em>&lt;br&gt;<em>L. plantarum</em>&lt;br&gt;<em>Lb. salivarius</em>&lt;br&gt;<em>Lb. vaginallis</em></td>
<td>ERY TET</td>
<td>Nawaz et al. (2011)</td>
<td></td>
</tr>
<tr>
<td>Jiang shui (fermented vegetable drink) pickled pepper</td>
<td><em>Lb. plantarum</em></td>
<td>TET</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fermented vegetable</td>
<td><em>Lb. salivarius</em></td>
<td>ERY TET PEN</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gochu-jangajji (Korean green chili pickled pepper)</td>
<td><em>Lb. plantarum</em></td>
<td>GEN KAN STR</td>
<td>Niu et al. (2019)</td>
<td></td>
</tr>
<tr>
<td>Jangajji (Korean pickled vegetable)</td>
<td><em>Lb. paraparallarum</em></td>
<td>KAN STR CIP</td>
<td>Son et al. (2018)</td>
<td></td>
</tr>
<tr>
<td>Kimchi</td>
<td><em>W. confusa</em>&lt;br&gt;<em>Lb. plantarum</em>&lt;br&gt;<em>Lb. mesenteroides</em>&lt;br&gt;<em>Lb. sakei</em>&lt;br&gt;<em>Lb. sakei</em>&lt;br&gt;<em>Lb. plantarum</em>&lt;br&gt;<em>Lb. plantarum</em></td>
<td>SPT&lt;br&gt;SPT VAN&lt;br&gt;AMP GEN KAN STR PMB PEN&lt;br&gt;GEN STR CIP&lt;br&gt;GEN STR KAN CIP</td>
<td>Dey et al. (2019)&lt;br&gt;Lee et al. (2016)&lt;br&gt;Won et al. (2020)&lt;br&gt;Lee et al. (2011)&lt;br&gt;Son et al. (2017)</td>
<td></td>
</tr>
<tr>
<td>Mustard pickles</td>
<td><em>Lb. plantarum</em>&lt;br&gt;<em>Lb. pentosus</em></td>
<td>PEN CEF CLO NOV VAN PMB RFP TET KAN GEN NEO STR</td>
<td>Huang et al. (2017)</td>
<td></td>
</tr>
<tr>
<td>Neera (fermented coconut palm nectar)</td>
<td><em>Lb. brevis</em>&lt;br&gt;<em>E. lactis</em>&lt;br&gt;<em>E. durans</em>&lt;br&gt;<em>Ln. lactis</em>&lt;br&gt;<em>E. faecium</em></td>
<td>KAN CLI AMP GEN ERY CHL</td>
<td>Somashekaparaiyah et al. (2019)</td>
<td></td>
</tr>
<tr>
<td>Table olives</td>
<td><em>Ln. mesenteroides</em>&lt;br&gt;<em>Lb. pentosus</em>&lt;br&gt;<em>Ln. pseudomesenteroides</em>&lt;br&gt;<em>Lb. plantarum</em>&lt;br&gt;<em>Lb. paraparallarum</em>&lt;br&gt;<em>Lb. paracasei</em> subsp. paracasei</td>
<td>KAN TET GEN AMP CHL VAN</td>
<td>Argyri et al. (2013)</td>
<td></td>
</tr>
<tr>
<td>Pickles</td>
<td><em>Lb. pentosus</em>&lt;br&gt;<em>Lb. plantarum</em>&lt;br&gt;<em>Lb. pentosus</em>&lt;br&gt;<em>Ln. mesenteroides</em>&lt;br&gt;<em>Lb. pentosus</em>&lt;br&gt;<em>Ln. pseudomesenteroides</em>&lt;br&gt;<em>Lb. pentosus</em>&lt;br&gt;<em>Lb. coryniformis</em></td>
<td>GEN VAN CLI FOT KAN NA STR&lt;br&gt;KAN GEN TET&lt;br&gt;STR VAN TEC TET SXT CXM CIP TET CLI&lt;br&gt;AMP ERY</td>
<td>Benitez-Cabello et al. (2019)&lt;br&gt;Botta et al. (2014)&lt;br&gt;Casado Muñoz et al. (2014)&lt;br&gt;Guantario et al. (2018)</td>
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</tbody>
</table>

(Continues)
TABLE 2 (Continued)

<table>
<thead>
<tr>
<th>Investigated product</th>
<th>Investigated microorganisms</th>
<th>Phenotypic resistance traits detected</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sauerkraut</td>
<td>Leuconostoc spp., Lactobacillus spp., Lb. plantarum</td>
<td>AMP CHL CLI GEN KAN STR TET ERY, KAN GEN VAN STR PMB</td>
<td>Tourret et al. (2018), Yeu et al. (2013)</td>
</tr>
<tr>
<td>Tempoyak (fermented durian fruit)</td>
<td>Fructobacillus duronis, Lb. plantarum</td>
<td>VAN NA SF SXT TMP CIP BC CEF STR TET KAN AMC CAR CHL AMP</td>
<td>Chuah et al. (2016)</td>
</tr>
<tr>
<td>Lb. plantarum, Lb. crispatus, Lb. reuteri, Lb. fermentum, Lb. pentosus</td>
<td></td>
<td>ERY VAN TET</td>
<td>Khalil et al. (2018)</td>
</tr>
</tbody>
</table>

Abbreviations: AMC, amoxicillin/clavulanic acid; AMK, amikacin; AMP, ampicillin; ATG, aztreonam; BC, bacitracin; CAR, carbenicillin; CEF, cephalothin; CHL, chloramphenicol; CIP, ciprofloxacin; CLI, clindamycin; CLO, cloxacillin; CMX, cefuroxime; ERY, erythromycin; FOT, cefotaxime; FOX, cefoxitin; GEN, gentamicin; KAN, kanamycin; LVX, levofloxacin; NA, nalidixic acid; NEO, neomycin; NOR, norfloxacin; NOV, novobiocin; PEN, penicillin G; PIR, pirlimycin; PMB, polymyxin B; RFP, rifampicin; SF, sulfafurazole; SPT, spectinomycin; STR, streptomycin; SXT, sulfamethoxazole/trimethoprim; TEC, teicoplanin; TET, tetracycline; TMP, trimethoprim; VAN, vancomycin.

Studies were largely based on examining the probiotic potential of LAB isolated from the products, as part of safety evaluation. The choice of antibiotics tested for susceptibility/resistance appears to be an arbitrary selection of clinically important antibiotics. Most of the studies assessed AR/susceptibility traits only phenotypically, through methods such as disk diffusion, agar dilution, broth microdilution, and E-test. However, a few studies additionally assessed the genotypic basis of resistance through polymerase chain reaction (PCR)-based methods (Casado Munoz et al., 2014; Gotcheva et al., 2018; Nawaz et al., 2011; Pan et al., 2011; Valledor et al., 2020).

Based on the studies included in this review, LAB isolated from spontaneously fermented plant-based foods have shown resistance to antibiotics from a wide range of antibiotic classes. These classes include aminoglycosides (amikacin, gentamicin, kanamycin, neomycin, streptomycin), cephalosporins (cephalothin, cefuroxime, cefotaxime, cefoxitin), quinolones (ciprofloxacin, levofloxacin, nalidixic acid, norfloxacin), phenicols (chloramphenicol), macrolides (erythromycin), glycopeptides (vancomycin, teicoplanin), lincosamides (clindamycin, pierlimycin), penicillins (amoxicillin, carbenicillin, cloxacillin, penicillin G), monobactams (aztreonam), aminocoumarins (novobiocin), polymyxins (polymyxin B), polypeptides (bacitracin), sulfonamides (sulfafurazole, sulfamethoxazole), aminocyclitols (spectinomycin), antifolates (trimethoprim), rifamycins (rifampicin), and tetracyclines (tetracycline).

It can be observed that certain AR traits are commonly present in LAB from spontaneously fermented plant-based foods. Whether or not these traits can be transferred to other nonpathogenic or pathogenic bacteria depends on the genetic basis underpinning the AR. Intrinsic resistance is the natural ability of the bacteria to thrive in the presence of antibiotic; it is unrelated to prior antibiotic exposure and does not result from gene acquisition (Fajardo et al., 2009). Such resistance is conveyed by naturally occurring genes located on the chromosome and has minimal transfer potential. However, genes responsible for intrinsic resistance could potentially be spread if flanked by insertion sequences (Das et al., 2020; Razavi et al., 2020).

In LAB, certain resistance traits may be generally classified as inherent to this bacterial group, although differences in tolerance may arise between species (Mathur & Singh, 2005). Antibiotics to which LAB appear to be naturally tolerant include bacitracin, cefoxitin, ciprofloxacin, sulphonamides, trimethoprim/sulfamethoxazole, vancomycin, and teicoplanin among others (Danielsen & Wind, 2003). Likewise, it was demonstrated elsewhere that LAB tend to tolerate aminoglycosides (amikacin, kanamycin, streptomycin, gentamicin), glycopeptides (vancomycin, teicoplanin), and quinolones (nalidixic acid, ciprofloxacin) (Coppola et al., 2005).

Aside from the wide range of intrinsic resistance displayed by LAB, this bacterial group also has the potential to acquire resistance to many relevant antimicrobials. Bacteria can acquire new genes either via mutation of existing genes (usually nontransferable) or by horizontal transmission (Devanga Ragupathi et al., 2019). The primary mechanism of HGT is thought to be conjugation. Conjugal horizontal transmission involves the direct transfer of mobile genomic elements (MGE)—potential carriers of AR genes, from donor to recipient bacteria (Gazzola et al., 2012). Tolerance to antimicrobials that given bacteria are normally susceptible to indicates that the trait might have been acquired via HGT. Such traits are likely to be subsequently transmitted to other bacteria.

Generally, LAB should be sensitive toward cell wall synthesis inhibitors (mostly penicillins including ampicillin and penicillin G) and protein synthesis inhibitors such as tetracyclines, chloramphenicol, erythromycin, and clindamycin (Federici et al., 2014). Specific resistance traits observed in LAB isolated from spontaneously fermented plant-based foods are discussed below.

3.1 Resistance to aminoglycosides

Resistance to one or more aminoglycoside antibiotic was reported in most of the articles herein reviewed. Tolerance to aminoglycosides in LAB is attributed to limited drug uptake originating from the lack of cytochrome-mediated electron transport and is regarded as non-transferable (Charteris et al., 2001). However, recently Jaimee and Halami (2016) have argued that the high-level resistance to aminoglycosides may be associated with potentially transmissible genes coding for aminoglycoside-modifying enzymes. So far, no studies have demonstrated the transfer of such resistance in nonpathogenic LAB, whereas
in enterococci, a filter-mating experiment resulted in the successful dissemination of streptomycin/gentamicin resistance gene from the donor to the recipient enterococci (Sparo et al., 2012). In the study by Somashekarahia et al. (2019), Enterococcus species isolated from Neera showed phenotypic resistance to aminoglycosides—kanamycin and gentamicin (Table 2). Molecular characterization may be needed to establish the potential transferability of the resistance.

3.2 | Resistance to vancomycin

In LAB, tolerance to glycopeptides such as vancomycin and teicoplanin is regarded as a common inherent trait (especially in Lactobacillus spp.), resulting from the alterations in the chemical structure of the peptidoglycan in the cell wall (Das et al., 2020; Juárez-Castelán et al., 2019). Vancomycin inhibits cell wall synthesis (Gotcheva et al., 2018).

However in enterococci, vancomycin resistance is not an intrinsic feature, being mediated by the van(A) gene located on a plasmid (DeLisle & Perl, 2003). Furthermore, there is some evidence demonstrating that van(A) may be passed from enterococci to other LAB, as in the case of Lactobacillus acidophilus, which acquired transposon-mediated van(A) in vivo in the mouse gut (Mater et al., 2008). Among the studies in this review, the presence of vancomycin resistance was reported only in lactobacilli. However, the intrinsic nature of the trait cannot be ascertained without genotypic analysis. In the study by Valledor et al. (2020), PCR analysis of the Enterococcus faecium strain isolated from boza was unable to detect various van genes (A, B, C, D, E, and G), and this corresponded with the vancomycin sensitivity phenotype. On the other hand, Gotcheva et al. (2018) detected van(A) in Lb. plantarum strains isolated from boza (Table 2).

3.3 | Resistance to quinolones

Casado Muñoz et al. (2014) reported a high percentage of ciprofloxacin resistance in Lb. pentosus strains isolated from naturally fermented olives. Molecular screening of the quinolone resistance-determining region disproved the presence of any mutated genes that would be flanked by insertion sequences, integrons, or transposons. Thus, the trait was considered intrinsic. Chromosomally encoded efflux pumps, such as NorA, MepA, and MdeA, were likely responsible for the intrinsic resistance, and the genes were widely detected (Casado Munoz et al., 2014). Similarly, other studies also suggest that quinolone tolerance is inherent to LAB, although the underlying mechanism is not well-understood (Hummel et al., 2007).

3.4 | Resistance to penicillins

It has been established that spontaneous chromosomal mutations may induce tolerance to penicillin antibiotics, resulting from reduced receptor binding (Danielsen & Wind, 2003). However, it may be also conferred by the plasmid-located bla(Z) gene as observed in Lb. plantarum (Aquilanti et al., 2007). Yet, because bla(Z) is seldom detected in LAB, there is a relatively low chance that resistances reported in this review are transmissible (Rizzotti et al., 2005). For instance, in Pan et al. (2011) 1%-4% of strains were ampicillin resistant and further genotypic screening was carried out to determine the characteristics of the resistance. No isolates were found to possess bla(Z) genes. This may therefore suggest that a different resistance mechanism was in place. Nevertheless, in other studies where penicillin resistance was observed, genotypic characterization would be required to exclude the possibility of bla(Z) presence.

3.5 | Resistance to tetracycline

At least 11 tetracycline resistance determinants coding either for ribosomal protection proteins or efflux pumps were previously identified in LAB (Lahtinen et al., 2009). These are often associated with MGE and thus are likely to be horizontally spread. For instance, the filter mating experiment by Gevers et al. (2003) led to the successful transfer of the tet(M) gene from lactobacilli to E. faecalis and Lc. lactis. Here, several studies reported the presence of tetracycline resistance in examined foods. In Nawaz et al. (2011), genotypic characterization indicated the presence of tet(M) and tet(S) tetracycline resistance determinants. Moreover, the subsequent filter mating of tet(M)-positive lactobacilli resulted in a transfer to E. faecalis, once more confirming the potential transferability of tetracycline resistance. However, tolerance to this antibiotic may be also mediated by efflux pump genes, which are non-transmissible due to their chromosomal location. This can be seen in the case of Casado Muñoz et al. (2014), where the high frequency of phenotypic tetracycline resistance in Lb. pentosus from olives was attributed to multidrug efflux pumps.

3.6 | Resistance to chloramphenicol

Tolerance to chloramphenicol in LAB might be associated with the plasmid-borne cat gene and although there is no empiric evidence on the transfer of cat between LAB species, studies suggest that it is feasible. For instance, Lin et al. (1996) described a high similarity of cat gene from Lactobacillus reuteri to cat from Staphylococcus aureus located on a plasmid. Therefore, it could be hypothesized that Lb. reuteri acquired the gene through horizontal transmission that brings the possibility of further spread from resistant LAB to other bacteria. In this review, some studies have reported chloramphenicol resistance. However, in the study by Pan et al. (2011), although chloramphenicol resistance in LAB from pickles was detected phenotypically, genotypic characterization did not detect the cat_pIP501 gene, implying the existence of different ARG genes in the investigated strains. It has been suggested recently that chloramphenicol resistance in LAB is in fact predominantly located on the chromosome, which would explain the lack of plasmid-encoded cat_pIP501 in analyzed isolates (Abriouel et al., 2015). Indeed, in earlier reports, the cat determinant has been detected on the chromosome in Lb. pentosus and Lb. plantarum (Hummel et al., 2007). These
chromosomal genes are not homologous to plasmid-encoded cat and are therefore considered nontransferable. Thus, it may be concluded that HGT of chloramphenicol resistance in LAB from fermented foods is possible, yet relatively rare (Abriouel et al., 2015).

3.7 | Resistance to erythromycin

Erythromycin resistance in LAB has been associated with the most frequently plasmid-located erm(B) gene (Fraqueza, 2015). Among the studies in the review where tolerance to erythromycin was reported, only Nawaz et al. (2011) performed a genotypic analysis that resulted in the identification of the erm(B) determinant. Furthermore, the detected gene was later acquired from resistant lactobacilli by E. faecalis in filter-mating trials. The transferability of the erm(B) gene in LAB is strongly supported by other literature. For instance, the trait was successfully transferred from Leuconostoc to E. faecalis strains on the surface of Monte Veronese cheese (Florez et al., 2016). Moreover, it has been demonstrated elsewhere that erm(B) from LAB can also be transmitted to nonlactic acid, pathogenic bacteria such as Listeria spp., which presents a major threat to potential antibiotic therapy (Toomey et al., 2009).

3.8 | Resistance to clindamycin

Macrolide–lincosamide–streptogramin (MLS) phenotype is a resistance mechanism that confers the resistance to lincosamides (clindamycin), macrolides (erythromycin), and streptogramin (Casado Muñoz et al., 2014). It is mediated by a mutation in erm genes resulting from continuous antibiotic exposure, and because erm genes are mostly plasmid located, there is a risk of spread (Pana, 2012). On the other hand, multidrug transporters in LAB such as LmrA might also be responsible for the resistance to clindamycin and up to 20 other antibiotics (Poelarends et al., 2002). Because these traits are located on the chromosome, they are classified as nontransmissible. In this review, clindamycin resistance was documented in some studies. Among them, some Lactobacillus and Enterococcus isolates were both erythromycin and clindamycin resistant, which indicates the possibility of the aforementioned MLS phenotype presence (Pana, 2012).

4 | CONCLUSION

Based on the findings of reviewed studies, spontaneously fermented plant-based foods may act as reservoirs of transferable AR genes and potentially contribute to the problem of AR. However, many studies tend to only carry out phenotypic characterization of AR traits. Molecular analysis is necessary to determine the genetic basis of observed phenotypes and distinguish between intrinsic and acquired/transferable traits. As highlighted, even some seemingly intrinsic traits might be transmissible. Transferability of AR genes from a donor to a recipient also needs to be demonstrated, through filter-mating experiments (Das et al., 2020). However, in vitro experiments may not adequately replicate the conditions in the gut, which may influence HGT (Polit et al., 2018).

Metagenomic sequencing approaches may be useful in examining the presence of mobile AR genes in spontaneously fermented foods, and have been applied in studies such as Leech et al. (2020), Toth et al. (2020), and Toth et al. (2021). However, these approaches would rely on robust databases that are representative of the wider microbial and genomic diversity that can occur in foods produced by spontaneous fermentation compared to those produced by starter culture (Leech et al., 2020).

Nevertheless, it can be inferred that there is a need to manage AR in spontaneously fermented plant-based foods. A strategy would be the employment of starter cultures in the manufacturing process. However, this could result in the loss of the unique organoleptic properties associated with natural fermentation (Capozzi et al., 2017). Recent advances enable the development of multistrain starters containing microbes native to the spontaneously fermented food. Therefore, a similar flavor could be obtained with these cultures (Maqueda et al., 2011).

Moreover, safety from an AR perspective could also be controlled by subjecting the fermented foods to heat or other treatments that reduce or eliminate microorganisms (Tamang et al., 2020). This would, however, also potentially impact organoleptic properties and certainly affect the live microbial content of these foods and health benefits that may accrue from live microorganisms. Overall, the presence of AR in fermented plant-based foods is a multifaceted problem that requires addressing not only the manufacturing but also broader issues such as antibiotic overuse in humans and livestock.

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CONFLICT OF INTEREST

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REFERENCES


faecium ST10Bz strain isolated from boza, a Bulgarian cereal-based beverage. Microorganisms. 8(10), 1474.


Won, S., Chen, S., Park, K. W., & Yoon, J. (2020). Isolation of lactic acid bacteria from kimchi and screening of Lactobacillus sakei ADM14 with anti-adipogenic effect and potential probiotic properties. LWT - Food Science and Technology, 126, 109296.


