Review Article

Antibiotic resistance of probiotic organisms and safety of probiotic dairy products

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Abstract: Intrinsic resistances to tetracycline, vancomycin and erythromycin are common in Lactobacillus species; however, resistance to streptomycin, clindamycin, gentamicin, oxacillin and lincosamide is also reported in these species. Resistant markers tet(W), tet(M) and erm(B) have been frequently detected in the resistant strains while van(A), Int(A) and tet(L) have also been found in some strains of Lactobacillus. Bifidobacteria are commonly resistant to tetracycline, streptomycin, erythromycin, gentamycin and clindamycin. Resistance genes van(A), tet(L) and tet(M) are often detected in Enterococcus. Reports suggest enterococci to transfer tet(M) to E. faecalis or Listeria strains and van(A) to commercial strain of Lactobacillus acidophilus. Streptococcus species are highly resistant to tetracycline, ciprofloxacin and aztreonam and tet(M) was detected in strains of dairy origin. Clinical cases of endocarditis, septicemia, bacteremia and septic arthritis due to the species of Lactobacillus, Saccharomyces, Leuconostoc, Pediococcus and Bifidobacterium have been reported in patients with some underlying medical conditions.

Keywords: Antibiotic resistance, probiotics, minimum inhibitory concentration

Introduction

The overwhelming use of antibiotics has played a significant role in the outspread/emergence of antibiotic resistance bacteria. Antibiotics added to animal-feed and given to livestock that are used as human food contribute to additional resistance. Reports suggest that commensal bacteria may act as potential reservoirs for antimicrobial resistance genes, hence bacteria used as probiotics for humans or animals should not carry any transferable antimicrobial resistance genes (von Wright, 2005; European Food Safety Authority-EFSA, 2008; The panel on additives and products or substances used in animal feed-FEEDAP, 2008). According to World Health Organization (WHO) global strategy for the containment of antimicrobial resistance (World Health Organization-WHO, 2001), the rate of emergence of antimicrobial resistance is expected to be increased by misuse of antibacterial substances. The resistant micro-organisms present in food products originating from animal source may cause infections in humans that are difficult to treat. A summary of risk factors for antibiotic resistance particularly relevant to, but not limited to, developing countries is outlined in Table 1.

The European Food Safety Authority (2005) has outlined a scheme based on the qualified presumption of safety (QPS) that involves the individual assessment and evaluation of acquired antibiotic resistance determinants in lactic acid bacteria (LAB).

According to the scheme, the members of the Lactococcus and Lactobacillus are most commonly given “generally regarded as safe” (GRAS) status, whilst members of the genera Streptococcus and Enterococcus and some other genera of LAB contain some opportunistic pathogens. Microorganisms used in animal feed in the European Union (EU) are mainly strains of Bacillus (B. cereus var. toyoii, B. licheniformis, B. subtilis), Enterococcus (E. faecium), Lactobacillus (L. acidophilus, L. casei, L. farciminis, L. plantarum, L. rhamnosus), Pediococcus (P. acidilactici), Streptococcus (S. infantarius), and yeast of Saccharomyces cerevisiae and Kluyveromyces species (Anadón et al., 2006).

Table 1. Human activities that exacerbate resistance (adapted from Okeke et al. (2005))

<table>
<thead>
<tr>
<th>Selective pressure</th>
<th>Dissemination of resistant organisms</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Appropriate antimicrobial use in chemotherapy</td>
<td>• Inadequate infection control in health-care institutions</td>
</tr>
<tr>
<td>• Use of a narrow repertoire of antimicrobials on most</td>
<td>• Shortfalls in hygiene, sanitation, and public health</td>
</tr>
<tr>
<td>patients</td>
<td>• Lack of surveillance and consequent late detection</td>
</tr>
<tr>
<td>• Antimicrobial misuse and abuse in human beings</td>
<td></td>
</tr>
<tr>
<td>• Agricultural antimicrobial use and misuse</td>
<td></td>
</tr>
<tr>
<td>• Use of poor quality antimicrobials</td>
<td></td>
</tr>
</tbody>
</table>

The guidelines updated by the FEEDAP Panel in 2008 are expected to eliminate the possibility of microorganisms from food chain to carry transmissible resistances genes. However, no such guidelines exist
concerning yeast resistance to antmycotics. As a result, the use of antimicrobial growth promoters such as avoparcin, carbadox and alaquindox has been banned in the EU since 2006. The emergence of vancomycin-resistant enterococci in food-animals is correlated with the use of avoparcin. Avoparcin is a glycopeptide that is used as a feed additive for adding the growth of animals that can cause spread of vancomycin-resistance from animals to humans (Wegener, 2003). Since the resistance in many cases is transmissible, non-pathogenic bacteria added into the food chain could act as a reservoir of resistance and transfer this trait to pathogens.

Types of antibiotic resistance

There are three types of resistance: natural (intrinsic or innate), acquired and mutational. According to FEEDAP (2008), strains carrying the acquired resistance due to acquisition of exogenous resistance genes are unacceptable for use as animal feed additives.

Resistance gene reservoir hypothesis

Colonic bacteria normally residing in colon act as reservoirs for resistance genes that can be acquired from ingested bacteria (Figure 1). According to reservoir hypothesis “commensal bacteria in the colon including those that could act as opportunistic pathogens and those that are truly non-pathogenic, exchange DNA with one another” (Salyers et al., 2008). The reservoir hypothesis suggests that antibiotic-resistant bacteria came into existence because of the selective pressures applied by antibiotic drugs (Table 1). ‘After antibiotic treatment, there is a decline in the populations of susceptible bacteria, naturally resistant bacteria begin to thrive, creating a reservoir of antibiotic-resistant bacteria’ (Salyers et al., 2004).

Methods for determining antibiotic resistance

Methods that are routinely used for testing antibiotic susceptibility of bacteria include Kirby-Bauer (disc diffusion) method, Stokes method, E-test (based on antibiotic diffusion), agar and broth dilution or agar dilution methods for the determination of minimum inhibitory concentration (MIC). The E-test (Epsilometer Testprinzip, Ellipse gradient test-AB Biodisk) is a popular quantitative technique for determining antimicrobial susceptibility. It is based on the combined concepts of in vitro dilution and diffusion tests. In the assay, ‘there is an immediate and effective release of the antimicrobials in a continuous exponential gradient when they are applied to an agar surface’ (Ribeiro et al., 2005). The technique is accurate and reproducible because of the stability of the antibiotics (Sader et al., 1994).

These methods have been tested and compared for different LAB and bifidobacteria. MICs can be determined by agar or broth dilution techniques by following the reference standards established by various authorities such as the Clinical and Laboratory Standards Institute (CLSI, USA), British Society for Antimicrobial Chemotherapy (BSAC, UK), Agence Francaise de Securite Sanitaire des Produits de Sante (AFFSAPS, France), Deutsches Institut für Normung e.V. (DIN, Germany) & ISC/WHO. FEEDAP has published guidelines regarding the testing procedures since 2001. FEEDAP requires the determination of the MICs of the most relevant antimicrobials for each bacterial strain that is used as a feed additive in order to eliminate the possibility of transmissible resistances.

Mayrhofer et al. (2008) tested 104 strains of L. acidophilus using broth microdilution, disk diffusion, and E-test. A good agreement was found between MICs from the broth microdilution method and the E-test method. Agar based methods such as E-test and agar disk diffusion were suggested as valid methods compared to the broth microdilution method. Blandino et al. (2008) found MICs as identical to those obtained with the E-test. Danielsen and Wind (2003) suggested that MICs can be used as a microbiological breakpoint when screening Lactobacillus strains for transferable resistance genes. For antimicrobial susceptibility testing of bifidobacteria, Mättö et al. (2007) suggested that the E-test on LAB susceptibility test medium supplemented with cysteine was useful. The swab and agar overlay gradient diffusion method was found to be reliable by Charteris et al. (2001) for antibiotic susceptibility testing of rapidly growing, facultative anaerobic lactobacilli, using MRS agar as test medium.

Figure1. The reservoir gene hypothesis. Bacteria residing in human colon can act as reservoir of resistant genes that can be acquired from ingested bacteria (adapted from Salyers et al. (2008))
Egervärn et al. (2007) found that results obtained with the E-test or the broth microdilution method for the assessment of antibiotic susceptibility of L. reuteri and L. fermentum strains (56 each) corresponded well with each other. This is supported by the study of Brown and Brown (1991) that showed a good correlation between MICs by the agar dilution and E-test methods. Turnidge and Paterson (2007) found that the distribution of MICs for wildtype strains of a single species was log-normal.

**Acquisition and spread of resistances**

The antibiotic resistance gene can be transferred by conjugation, transduction or transformation (Figure 1). At present, reports regarding the spread of antibiotic resistance among LAB and bifidobacteria suggest that resistant strains from human and animal colons are rather common, that confirms the transfer of resistances between commensal organisms in the complex ecosystem of gastro-intestinal tract (GIT) (Ammor et al., 2007). There is a general concern that such microbes may harbor genes that may contribute to opportunistic infections (Tompkins et al., 2008).

Theoretical risks that have been raised with respect to the use of probiotics in humans include the potential for transmigration and colonization and an adverse immunological effect. There is also a potential for antibiotic resistance transfer within the gastrointestinal tract from commensal or probiotic bacteria to other bacteria or potential pathogen (Snydman, 2008).

Starter cultures used in food products could also be a source of spread of antibiotic resistance. Hence, strains intended for use in feed and food systems should be systematically monitored for resistance in order to avoid their inclusion in starters and probiotic preparations (Ammor et al., 2007). Two genes namely, transposon-associated tet(M) gene and plasmid-carried tet(L) gene that mediate 2 different tetracycline resistance mechanisms have been described in L. sakei Rits 9 strain isolated from Italian Sola cheese made from raw milk (Ammor et al., 2008). Tetracycline resistance gene tet(K) in 5 Staphylococcus isolates used as meat starter cultures were detected by Kastner et al. (2006). In a recent report where the gene tet(M) of L. plantarum isolated from pork abattoir was transferred to Lc. lactis BU-2-60 and to E. faecalis JH2-2 (Toomey et al., 2010).

**Antibiotic resistance in LAB, Bifidobacterium and Bacillus spp.**

In the EFSA guidelines (The panel on additives and products or substances used in animal feed- FEEDAP, 2008), the MICs for relevant antimicrobials have been set for the following genera (and in some cases individual species): Lactobacillus, Lactococcus, Streptococcus thermophilus, Pediococcus, Leuconostoc, Enterococcus, Propionibacterium, Bifidobacterium and Bacillus. These genera also cover the recent QPS lists for bacteria, and consequently the FEEDAP approach can be directly applied.

LAB are intrinsically resistant to many antibiotics. In many cases, resistance is not always transmissible, and the species are also sensitive to many clinically used antibiotics in the case of a LAB-associated opportunistic infection. Therefore no particular safety concern is associated with intrinsic type of resistance. Plasmid-associated antibiotic resistance, which occasionally occurs, may spread resistance to other more harmful species and genera.

Using the disc diffusion method, antibiotic resistance among 187 isolates from 55 European probiotic products showed that 79% of the isolates were resistant against kanamycin and 65% of the isolates were vancomycin resistant. Remaining resistances were in the order of tetracycline (26%), penicillin G (23%), erythromycin (16%) and chloramphenicol (11%). Overall, 68.4% of the isolates showed resistance against multiple antibiotics including intrinsic resistance (Temmerman et al., 2003). In a study by Toomey et al. (2010), intrinsic streptomycin resistance was observed in lactobacilli, streptococci, lactococci and Leuconostoc spp.

Several studies have been carried out to test the antimicrobial susceptibility of different probiotic and LAB in different food products but only some of these have demonstrated the genetic basis of these resistances. Also, the data is available regarding antimicrobial resistance pattern in food-associated LAB such as lactobacilli but it is mostly based on non-standardized methodologies and/or has been obtained for only a limited number of strains (Huys et al., 2008). Studies regarding antimicrobial testing of different LAB, bifidobacteria and Bacillus strains have been summarized in Table 2 and discussed below.

**Lactobacillus**

Lactobacilli display a wide range of antibiotic resistance naturally, but in most cases antibiotic resistance is not of the transmissible type. Lactobacillus strains with non-transmissible antibiotic resistance do not form a safety concern. In a study by Danielsen and Wind (2003), out of 62 strains tested for antibiotic susceptibility, 6 strains
Table 2. Antibiotic resistance and safety implication of different LAB, bifidobacteria and *Bacillus* spp.

<table>
<thead>
<tr>
<th>Probiotic studied</th>
<th>Antibiotics found to be resistant*</th>
<th>Resistance gene</th>
<th>Acquisition or spread of resistance/origin/source of probiotic</th>
<th>Method used for antibiotic resistance analysis</th>
<th>References</th>
<th>Implication (Safety)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>L. farciminis</em> BFE 7438</td>
<td>Cip, Gen, Str</td>
<td>-</td>
<td>Intrinsic</td>
<td>3 strains were newly developed and 2 from commercial probiotic product. (Germany)</td>
<td>E-test, PCR, Southern hybridization, filter mating experiment</td>
<td>Hummel et al. (2007)</td>
</tr>
<tr>
<td><em>L. salivarius</em> BFE 7441</td>
<td>Cip, Ery, Gen, Str</td>
<td><em>ermB</em></td>
<td>Chromosomal</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>L. rhamnosus</em> BFE 7442</td>
<td>Cip, Gen, Str</td>
<td>-</td>
<td>Intrinsic</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>E. acidophilus</em> BFE 7444</td>
<td>Cip, Gen, Str</td>
<td>-</td>
<td>Intrinsic, inactive cat genes</td>
<td></td>
<td></td>
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<tr>
<td><em>L. casei</em> BFE 7445</td>
<td>Cip, Gen, Str</td>
<td>-</td>
<td>Intrinsic</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>L. paracasei</em></td>
<td>Gen, Van</td>
<td>-</td>
<td>Intrinsic (Van)</td>
<td></td>
<td></td>
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<tr>
<td><em>E. faecium</em></td>
<td>Gen, Van</td>
<td>-</td>
<td>Intrinsic (Van), Atypical (Ery)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>L. salivarius</em></td>
<td>Ery, Van</td>
<td>-</td>
<td>Intrinsic</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>L. acidophilus</em></td>
<td>Gen</td>
<td>-</td>
<td>Intrinsic</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>S. thermophilus</em></td>
<td>Cip</td>
<td>-</td>
<td>Intrinsic</td>
<td></td>
<td></td>
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<tr>
<td><em>L. rhamnosus</em> HN001 (DR20&lt;sup&gt;TM&lt;/sup&gt;)</td>
<td>Fus, Gen, Kan, Nal, Neo, Pol, Str, Van</td>
<td>-</td>
<td>Intrinsic (contain plasmids but antibiotic resistance is not linked)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>L. rhamnosus</em> HN007</td>
<td>Fus, Kan, Nal, Neo, Pol, Van,</td>
<td>-</td>
<td>Intrinsic</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>B. lactis</em> HN019 (DR10&lt;sup&gt;TM&lt;/sup&gt;)</td>
<td>Clo, Gen, Kan, Nal, Neo, Pol, Str</td>
<td>-</td>
<td>Intrinsic</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>L. acidophilus</em> HN017</td>
<td>Fus, Kan, Nal, Pol, Str</td>
<td>-</td>
<td>Intrinsic</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>L. plantarum</em> HN045</td>
<td>Clo, Fus, Gen, Kan, Nal, Neo, Pol, Str, Van</td>
<td>-</td>
<td>Intrinsic</td>
<td></td>
<td></td>
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</tr>
<tr>
<td><em>L. rhamnosus</em> GG</td>
<td>Clo, Fus, Gen, Kan, Nal, Neo, Pol, Str, Van</td>
<td>-</td>
<td>Intrinsic</td>
<td></td>
<td></td>
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</tr>
<tr>
<td><em>E. acidophilus</em> LA-1</td>
<td>Fus, Gen, Kan, Nal, Neo, Pol, Str</td>
<td>-</td>
<td>Intrinsic</td>
<td></td>
<td></td>
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</tr>
<tr>
<td><em>B. lactis</em> Bb12</td>
<td>Fus, Gen, Kan, Nal, Neo, Pol, Str, Van</td>
<td>-</td>
<td>Intrinsic</td>
<td></td>
<td></td>
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</tr>
<tr>
<td><em>B. lactis</em> HN049</td>
<td>Clo, Fus, Gen, Kan, Nal, Neo, Pol, Str</td>
<td>-</td>
<td>Intrinsic</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>B. lactis</em> HN098</td>
<td>Clo, Fus, Gen, Kan, Nal, Neo, Pol, Str</td>
<td>-</td>
<td>Intrinsic</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>B. breve</em> (Yakult)</td>
<td>Str</td>
<td><em>rpsL</em> gene</td>
<td>Atypical</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>L. rhamnosus</em> HN001 and HN067, <em>L. rhamnosus</em> HN071 and HN093, <em>L. acidophilus</em> HN017 and HN019, <em>B. lactis</em> HN019</td>
<td>Fus, Gen, Kan, Nal, Neo, Pol, Str, Van</td>
<td>-</td>
<td>Intrinsic</td>
<td>Commercial strain, Fonterra Research Centre Culture Collection (New Zealand, USA)</td>
<td>Disk diffusion method, PCR and sequencing experiment</td>
<td></td>
</tr>
<tr>
<td><em>B. breve</em> (Yakult)</td>
<td>Str</td>
<td><em>rpsL</em> gene</td>
<td>Atypical</td>
<td>Culture Collection Research Laboratory of Yakult Central Institute for Microbiological Research (Tokyo, Japan)</td>
<td>Broth microdilution method, PCR and sequencing experiment</td>
<td>Kiwaki and Sato (2009)</td>
</tr>
<tr>
<td>Probiotic Organism</td>
<td>Gen, Kan, Nal, Ofr, Str, Tet, Tob (confirmed by E-test)</td>
<td>Antibiotic Resistance Determinants</td>
<td>Methodology</td>
<td>Reference</td>
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<tr>
<td>B. lactis DSM 10140</td>
<td>Gen, Kan, Nal, Ofr, Str, Tet, Tob (confirmed by E-test)</td>
<td>tet(W)</td>
<td>Disk diffusion, E-test, microarray and membrane hybridization techniques, PCR, partial sequencing methods and filter mating experiments</td>
<td>Kastner et al. (2006)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>L. reuteri SD 2112 (ATCC 55730)</td>
<td>Ctx, Fus, Kan, Lin, Met, Nal, Nal, Osa, Pen, Str, Tet, Tob, Van</td>
<td>tet(W), Ind(A)</td>
<td>Human origin. One strain obtained from ATCC and other from commercial tablet (Switzerland)</td>
<td>Disk diffusion method, serial antibiotic dilution procedure</td>
<td>Sorokulova et al. (2008)</td>
<td></td>
</tr>
<tr>
<td>L. rhamnosus strain GG (ATCC 53103)</td>
<td>Fus, Kan, Nal, Nal, Tob, Osa, Str, Van</td>
<td>vanA, vanB, vanC, mef (detected by microchip hybridization)</td>
<td>Phenotypic</td>
<td>Human (Switzerland)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bacillus subtilis VKPM B2335</td>
<td>Oxa</td>
<td>n/a</td>
<td>Ukrainian Collection of Microorganisms (France, Russia, UK)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bacillus licheniformis VKPM B2336</td>
<td>Amp, Cet, Cex, Clh, Ctx, Met, Oxa, Oxa</td>
<td>n/a</td>
<td>B. subtilis strain may be considered as non-pathogenic and safe for human consumption</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>L. acidophilus</td>
<td>Apr, Cep, Cip, Col, Gen, Kan, Nal, Neo, Sul, Tri</td>
<td>gyrA (Cip)</td>
<td>Intrinsic</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L. rhamnosus</td>
<td>Apr, Cep, Cip, Col, Gen, Kan, Nal, Neo, Spe, Sul, Tri, Van</td>
<td>-</td>
<td>Intrinsic</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L. plantarum</td>
<td>Amp, Cip, Anch, Gen, Kan, Nal, Neo, Spe, Sul, Tet, Tri, Van</td>
<td>gyrA (Cip), aadE (Str)</td>
<td>Intrinsic</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L. casei</td>
<td>Amp, Apr, Cep, Cip, Col, Gen, Kan, Nal, Neo, Spe, Sul, Tet, Tri, Van</td>
<td>-</td>
<td>Intrinsic</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L. paraginplanum</td>
<td>Amp, Apr, Cep, Cip, Col, Gen, Kan, Nal, Neo, Spe, Sul, Tet, Tri, Van</td>
<td>gyrA (Cip), tet(S)</td>
<td>Intrinsic</td>
<td></td>
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</tr>
<tr>
<td>B. longum</td>
<td>Amp, Cep, Col, Gen, Kan, Nal, Neo, Osa</td>
<td>aadE (Str)</td>
<td>Intrinsic</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>B. bifidum</td>
<td>Apr, Cep, Col, Gen, Kan, Nal, Neo, Osa, Osa, Osa</td>
<td>gyrA (Cip)</td>
<td>Intrinsic</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L. rhamnosus</td>
<td>Apr, Cep, Cip, Col, Gen, Kan, Nal, Neo, Tri</td>
<td>-</td>
<td>Intrinsic</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L. rhamnosus</td>
<td>Apr, Cep, Cip, Col, Gen, Kan, Nal, Neo, Spe, Sul, Tri, Van</td>
<td>-</td>
<td>Intrinsic</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L. paracasei</td>
<td>Apr, Cep, Cip, Col, Gen, Kan, Nal, Neo, Str, Sul, Tri, Van</td>
<td>aph(3’)-III (Kan, Neo), aadA (Str)</td>
<td>Intrinsic</td>
<td>Human feces, Europe (Denmark)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>L. casei</td>
<td>Apr, Cep, Cip, Col, Gen, Kan, Nal, Neo, Spe, Sul, Tri, Van</td>
<td>aph(3’)-III (Kan, Neo), aadA, aadE (Str), aadD (Spe)</td>
<td>Intrinsic</td>
<td>Semi-hard cheese, Europe (Denmark)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bifidobacterium sp</td>
<td>Apr, Cip, Col, Gen, Kan, Nal, Neo, Tri</td>
<td>-</td>
<td>Intrinsic</td>
<td>Human feces, Europe (Denmark)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>E. faecium</strong></td>
<td>Phenotypic and genetic resistances</td>
<td>Broth microdilution, filter mating experiments, PCR-based detection of resistant genes, PGFE and (GTG),* PCR, multiplex PCR</td>
<td>Vankerekoven et al. (2008)</td>
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<tr>
<td><em>erm(B)</em>, <em>cat</em>(pC194), <em>car</em>IP501), <em>aad(E), aad(E)</em>-aph(A)</td>
<td>6 strains from Probiotic products, 2 strains as probiotics for human and animal consumption comes from fecal flora (Sweden; 1968) and dairy product, cheese (Italy), 2 strains used commercially as a probiotic for human consumption. (Belgium, Germany)</td>
<td>Resistant to fusidic acid was demonstrated in a high percentage (92%) of isolates. Two probiotic isolates were phenotypically resistant to erythromycin, one of which contained an <em>erm(B)</em> gene that was not transferable to enterococcal recipients.</td>
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</table>

<table>
<thead>
<tr>
<th><strong>L. reuteri DSM 20016</strong></th>
<th>Cip, Gen, Oxa, Sul/Tri, Van,</th>
<th>n/a</th>
<th>n/a</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>L. reuteri 1-1 (ATCC 55149)</strong></td>
<td>Cip, Gen, Oxa, Sul/Tri, Van</td>
<td>n/a</td>
<td>n/a</td>
</tr>
<tr>
<td><strong>L. reuteri 1065 (ATCC 55168)</strong></td>
<td>Cip, Gen, Oxa, Sul/Tri, Van</td>
<td>n/a</td>
<td>n/a</td>
</tr>
<tr>
<td><strong>L. reuteri SD2112 (ATCC55730)</strong></td>
<td>Am, Cep, Cip, Gen, Oxa, Sul/Tri, Tet, Van</td>
<td>n/a</td>
<td>Plasmid borne</td>
</tr>
<tr>
<td><strong>L. reuteri 11284 (ATCC55148)</strong></td>
<td>Cip, Gen, Oxa, Sul/Tri, Tet, Van</td>
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<tr>
<td><strong>L. rhamnosus GG (ATCC 53103)</strong></td>
<td>Cip, Gen, Oxa, Sul/Tri, Van</td>
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<td>n/a</td>
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</table>

<table>
<thead>
<tr>
<th><strong>L. reuteri ATCC 55730 (SD2112)</strong></th>
<th>Amp, Lin, Tet</th>
<th><em>tet(W)</em>, <em>Ins(A)</em></th>
<th>Biogaia AB, Human milk. (Sweden)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Lactobacillus strain GG</strong></td>
<td>Gen, Mez, Tri/Sul, Str, Van</td>
<td>n/a</td>
<td>Adult human feces. Prof. Range Fonden (Panove Partner AB, Arla group, Stockholm Sweden). (Ireland)</td>
</tr>
<tr>
<td><strong>L. rhamnosus</strong></td>
<td>Gen, Mez, Tri/Sul, Str, Van</td>
<td>n/a</td>
<td>Dairy product, NCFB(NCIMB Ltd., Aberdeen, Scotland).</td>
</tr>
</tbody>
</table>

---

* Amp, Ampicillin; Apr, Apramycin; Ctx, Cefotaxime; Cef, Cefotaxime; Cep, Cefpodoxime; Cet, Ceftriaxon; Cep, Cephalothin; Chl, Chloramphenicol; Cip, Ciprofloxacin; Cl, Clindamycin; Co, Cloxacillin; Col, Colistin; Ery, Erythromycin; Fus, Fusidic acid; Gen, Gentamicin; Kan, Kanamycin; Lin, Lincomycin; Met, Methicillin; Mez, Metronidazole; Nan, Naldixid acid; Neo, Neomycin; Nit, Nitrofurantoin; Ofl, Ofloxacin; Oxa, Oxacillin; Pan, Penicillin; Pol, Polymyxin B; Spi, Spectinomycin; Str, Streptomycin; Sul, Sulphamethoxazole; Tetr, Tetracycline; Tob, Tobramycin; Tri, Trimethoprim; Van, Vancomycin
of lactobacilli showed transferable resistance genes on the basis of their resistance to chloramphenicol, erythromycin/clindamycin, and tetracycline. One strain of *L. rhamnosus* exhibited an elevated MIC for oxacillin. The genetic basis of this kind of resistance was proposed to be either due to mutations in the penicillin-binding proteins or due to the presence of a β-lactamase.

In the study of D’Aimmo et al. (2007), lactobacilli were found resistant to nalidixic acid, aztreonam, cycloserin, kanamycin, metronidazole, polymyxin B, spectinomycin and susceptible to rifampicin, bacitracin, clindamycin, erythromycin, novobiocin and penicillin. High resistance to nalidixic acid was found among all strains of *L. acidophilus* and *L. casei* whereas *L. casei* also demonstrated high resistance to aztreonam, cycloserine, polymyxin B and vancomycin.

MICs of 16 antimicrobials for 473 isolates of LAB comprising of the genera *Lactobacillus*, *Pediococcus* and *Lactococcus* were determined by Klare et al. (2007). The results suggested that majority of LAB were susceptible to penicillin, ampicillin, ampicillin/subactam, quinupristin/dalfopristin, chloramphenicol and linezolid. LAB exhibited a broad or partly species-dependent MIC profile of trimethoprim, trimethoprim/sulfamethoxazole, vancomycin, teicoplanin and fusidic acid. Noticeably, 3 probiotic *Lactobacillus* strains were highly resistant to streptomycin. Although erythromycin, clindamycin, and oxytetracycline possessed high antimicrobial activities, 17 *Lactobacillus* isolates were resistant to one or more of these antibiotics. Eight of them, including 6 probiotic and nutritional cultures possessed *erm(B)* and/or *tet(W)*, *tet(M)* or unidentified members of the *tet(M)* group. High resistance against streptomycin has also been reported in 1 strain of *Lactobacillus* isolated from Norwegian dairy product (Katla et al., 2001).

In the study of Huys et al. (2008), genotypically unique 65 strains of *L. paracasei* and *L. casei* were assayed for antibiotic resistance with broth microdilution and E-test assays using the LAB susceptibility test medium. In both methodologies, strains appeared uniformly susceptible to ampicillin and clindamycin but exhibited natural resistance to streptomycin and gentamicin. Three *L. paracasei* strains from cheese displayed acquired resistance to tetracycline (MIC ≥ 32 μg per mL) and/or erythromycin (MIC >16 μg per mL), which were linked to the presence of a *tet(M)* or *tet(W)* gene and/or an *erm(B)* gene, respectively. In the study of Kastner et al. (2006), *L. reuteri* SD 2112 has been shown to harbor tetracycline resistance gene *tet(W)* (residing on a plasmid) and the lincosamide resistance gene *lnu(A)*. Two plasmids carrying *tet(W)* tetracycline, and *lnu(A)* lincosamide resistance genes were also identified by Rosander et al. (2008) in a commercial strain of *L. reuteri* ATCC55730.

Both a transposon-associated *tet(M)* gene, and plasmid-carried *tet(L)* gene presenting 2 different tetracycline resistance mechanisms have been characterized in *L. sakei* Rits 9 strain isolated from Italian Sola cheese made from raw milk (Ammor et al., 2008). The 2 resistance determinants conferred different levels of resistance and their expression is induced by different tetracycline concentrations.

In a recent double blind clinical study by Egervárn et al. (2010), the transferability of tetracycline resistance gene *tet(W)* from *L. reuteri* to human gut flora was investigated particularly to fecal enterococci, bifidobacteria and lactobacilli. *L. reuteri* ATCC 55730 harboring a plasmid-encoded *tet(W)* gene was consumed by 7 subjects and an equal number of subjects consumed *L. reuteri* DSM 17938. No *tet(W)-reuteri* signal was produced from any of the DNA samples and thus evidence of gene transfer to entrococci, bifidobacteria and lactobacilli during intestinal passage of the probiotic strain was not found under the conditions tested.

In the study of Gfeller et al. (2003), *L. fermentum* ROT1 isolated from a raw milk dairy product was found resistant to novobiocin, tetracycline, erythromycin and dalfopristin. A chromosomal tetracycline-resistance determinant *tet(M)* was identified in the strain and a 19,398-bp plasmid (pLME300), present in several erythromycin-resistant strains of *L. fermentum*, was isolated and completely sequenced.

Several species of *Lactobacillus* including *L. rhamnosus* and *L. casei* are intrinsically resistant to vancomycin. There is an underlying possibility that vancomycin resistance could be transferred to other bacteria but there are no such reports to date. However, the transfer of vancomycin resistance (*vanA*) from enterococci to a commercial *L. acidophilus* strain was observed *in vitro* and *in vivo* in mice (Mater et al., 2008). In a study by Klein et al. (2000), all *Lactobacillus* strains namely 6 *L. reuteri* strains (ATCC 55730, ATCC 55149, ATCC 55148, ATCC 53608 and DSM 20016[3]) and 1 *L. rhamnosus* strain GG (ATCC 53103) were found resistant to vancomycin but susceptible to a broad range of antibiotics. Four of the *Lactobacillus* strains (including *L. rhamnosus* strains) did not harbor any plasmid but 2 of them showed 5 and 6 plasmid bands, respectively. None of the strains possessed the *vanA*, *vanB* or *vanC* gene. The findings established the safety of the *Lactobacillus* strains for use as probiotics concerning their vancomycin resistance (Klein et al., 2000). Zhou
et al. (2005) found 3 *L. rhamnosus* strains (HN001, HN067 and GG) resistant to vancomycin and of the 4 new probiotic strains namely, *L. rhamnosus* HN001, HN067, *L. acidophilus* HN017 and *B. lactis* HN019, only *L. rhamnosus* HN001 contained plasmids. A plasmid-free derivative of the strain had the same antibiotic susceptibility profile as the parent strain.

Charteris et al. (2001) found vancomycin resistance in all tested strains of *Lactobacillus* strain GG and 11 closely related, rapidly growing, facultatively anaerobic, potentially probiotic *L. rhamnosus* strains. Moreover, these strains were also resistant to co-trimoxazole, metronidazole, gentamicin, and streptomycin but sensitive to penicillin G, ampicillin, rifampicin, tetracycline, chloramphenicol, and erythromycin. Antibiotic susceptibility pattern of the strains derived from 10 Italian probiotic products was determined by Blandino et al. (2008). Intrinsic resistance to vancomycin was confirmed for *L. paracasei*, *L. salivarius* and *L. plantarum*, and atypical resistance to erythromycin was detected in 1 strain of *L. salivarius* according to FEEDAP and CLSI breakpoints (MIC ≥8 mg per L) (Blandino et al., 2008).

In the study of Toomey et al. (2010), all strains of *Lactobacillus* spp. including *L. paracasei*, *L. reuteri* and *L. curvatus*, except *L. plantarum* were resistant to erythromycin containing *erm(B)* and *msrA/B* genes. Tetracycline resistance was demonstrated by only *L. plantarum* determined by *tet(M)* gene and *Leuconostoc mesenteroides* spp. containing *tet(S)* gene, respectively. *L. plantarum* was also intrinsically resistant to vancomycin, however no vancomycin gene markers were found in *Lactobacillus* species. Intrinsic streptomycin resistance was observed in lactobacilli besides streptococci, lactococci and *Leuconostoc* species. In another report, *L. reuteri* 12002 of African origin, isolated from pig feces and used as probiotic intervention studies was found to harbor the *erm(B)* gene that could be transferred in vitro to enterococci. Twelve probiotic isolates of European origin demonstrated high prevalence of phenotypic resistance for aminoglycosides (Ouoba et al., 2008).

In a study by Egervärv et al. (2007), *L. reuteri* and *L. fermentum* (56 strains of each) were assessed for antibiotic susceptibility using an E-test kit and a broth microdilution method. *L. fermentum* has shown an uniform distribution for tested antibiotics including ampicillin, tetracycline, erythromycin, clindamycin, streptomycin, and gentamicin, whereas *L. reuteri* strains displayed bimodal distribution of MICs or above the test range for erythromycin, clindamycin, kanamycin, vancomycin, tetracycline, and trimethoprim. *L. reuteri* strains with high MICs for both ampicillin, and tetracycline exhibited genetic relatedness and 6 strains with high MICs for both erythromycin and clindamycin were also closely related.

**Bifidobacterium**

In the study of Mättö et al. (2007), human or probiotic associated *Bifidobacterium* species (203 strains) showed high MIC for tetracycline i.e. ≥16 mg per mL (prevalence of 4-18%) that was attributed to the presence of tet gene, where *tet(W)*, and *tet(O)* were detected. Occasional erythromycin (2%) and/or clindamycin (5%) resistant strains were found, while the strains were uniformly susceptible to ampicillin and vancomycin. MICs of tetracyclines were determined for 86 human *Bifidobacterium* isolates and 3 environmental strains. The *tet(O)* gene was absent in these isolates. *tet(W)*, and *tet(M)* were found in 26, and 7%, respectively, of the *Bifidobacterium* isolates, and one isolate contained both genes. Chromosomal DNA hybridization showed that there was one chromosomal copy of *tet(W)*, and/or *tet(M) (Aires et al., 2007). The tetracycline resistance gene *tet(W)* in the probiotic culture of *B. lactis* DSM 10140 was detected by Kastner et al. (2006).

Kiwaki and Sato (2009) determined the MICs of 17 antimicrobials for 26 *Bifidobacterium breve* strains of various origins by broth microdilution. MIC distributions for 17 antimicrobials were unimodal except streptomycin and tetracycline, in which it was bimodal. The probiotic *B. breve* strain Yakult showed intrinsic susceptibility to all antimicrobials except streptomycin to which the strain showed an atypically higher MIC of >256 μg per mL. The resistance of *B. breve* strain Yakult to streptomycin was caused by a chromosomal mutation of the *rps(L)* gene for ribosomal protein S12, and thus unlikely to be transferred to other microorganisms.

In another study by Blandino et al. (2008), the strains of *Bifidobacterium* were found susceptible to ampicillin, cefotaxime and erythromycin. In the study of Mättö et al. (2007), *Bifidobacterium* strains displayed generally high MICs for streptomycin and gentamicin suggesting intrinsic resistance. D’Aimmo et al. (2007) found that bifidobacteria were resistant to aminoglycosides, cycloserine, nalidixic acid and strongly resistant to kanamycin, polymixin B, and aztreonam (MIC90 = 1000 μg per mL).

**Enterococcus**

Members of *Enterococcus* contain some
opportunistic pathogens, hence, it is debated as to whether these organisms could be used as probiotics. Several studies have examined the antibiotic resistance profile, and evaluated the transferability of the resistance determinants to other microorganisms. Rizzotti et al. (2009) studied the diversity and transferability of tetracycline gene tet(M) of 20 enterococci belonging to species of *E. faecalis* (12 strains), *E. faecium* (4), *E. durans* (2), *E. hirae* (1), and *E. mundtii* (1) originating from swine meat. The gene tet(L) was observed in the 50% of the strains and tet(M) was found correlated with a transposon of the Tn916-1545 family. Moreover 50% of enterococcal strains showed the ability to transfer tet(M) gene to *E. faecalis* or *Listeria innocua* strains, which affirms the spread of tetracycline resistance in enterococci to potentially pathogenic bacteria occurring in food chain.

Mater et al. (2008) observed the transfer of vancomycin resistance (*vanA*) from enterococci to a commercial strain of *L. acidophilus in vitro* and *in vivo* in mice. The transconjugants were obtained in high frequency and were capable of persisting in the digestive environment of mice. Since the same transfer is expected to occur in human digestive tract, it raises a safety concern regarding the use of probiotics comprising lactobacilli in either immunocompromised individuals or during antibiotic therapy. In vancomycin resistant *E. faecium* isolates collected from Michigan hospitals, the location of *vanA* genes was found on both plasmid and chromosome that suggests the possibility of transposon dissemination among these isolates (Thal et al., 1998).

Regarding the prevalence of antimicrobial resistance of enterococcal strains in different environments, the frequency of various antimicrobial resistances was much lower in food isolates in comparison to clinical strains (Abriouel et al., 2008). Similar findings were reported by Blandino et al. (2008) where *E. faecium* derived from probiotic product from Italy was susceptible to all the tested antibiotics including vancomycin, ampicillin, cefaclor, cefotaxime, erythromycin, ciprofloxacin and gentamicin. However, in the Moroccan food isolates studied by Valenzuela et al. (2008), the frequency of antimicrobial resistance was remarkably high. The resistance profiles of *E. faecalis* were different from those of *E. faecium*, tetracycline resistance being typical to the former and erythromycin resistance to the latter. Similarly, in the study of (Devirgiliis et al., 2010), high MIC values for tetracycline were found among 16 strains of *E. faecalis* isolated from Italian fermented dairy products. The presence of tet(M) was demonstrated by the resistant strains that pose a potential risk of horizontal transfer of the resistant gene among other food borne commensal bacteria.

*E. faecalis* strains isolated from Irish pork and beef abattoirs were susceptible to vancomycin, however, 4 of 10 strains of *E. faecium* were resistant to vancomycin but no corresponding genetic determinants for this phenotype were detected (Toomey et al., 2010). *E. faecium* isolated from an European probiotic product was found resistant to vancomycin using disc diffusion method but later it was confirmed by broth dilution and PCR that the isolates were vancomycin sensitive (Temmerman et al., 2003). Susceptibility of 128 isolates of *E. faecium* used as probiotic cultures was tested for 16 antimicrobial agents using broth microdilution. Two isolates were phenotypically resistant to erythromycin, 1 of which contained an *erm*(B) gene that was not transferable to enterococcal recipients (Vanerkckhoven et al., 2008). In the study of Tompkins et al. (2008), MIC values for *E. faecium* R0026 for 17 antimicrobials were below the break-point values published by EFSA. The strain used in different commercial probiotic products was susceptible to gentamicin, streptomycin and vancomycin.

Use of growth promoters creates a major food animal reservoir of resistant bacteria, with a potential for spread to humans through food intake or by contact with animal (Wegener, 2003). Butaye et al. (2000) tested 76 *E. faecium* strains originated from poultry meat, cheese and raw pork for their susceptibility and resistance to growth-promoting antibacterials used in animals and antibiotics used therapeutically in humans. High-level of streptomycin resistance was observed in strains of all origins, though infrequently but the strains isolated from poultry meat showed more resistances against bacitracin, virginiamycin, narasin, tylosin (a macrolide antibiotic), ampicillin, glycopeptides avoparcin and vancomycin.

*Enterococcus* species can be found in the same habitat as of the *Listeria* species. Hence, these can be important sources of transferring antibiotic resistance through mobile genetic elements such as transposons to *Listeria*. A horizontal spread of resistance to *Listeria* spp. could be possible in some steps of the food production (Rizzotti et al., 2009).

**Streptococcus**

A strain of *S. thermophilus* isolated from a probiotic product available in Italy was found resistant only to ciprofloxacin among the tested antibiotics (Blandino et al., 2008). D’Aimmo et al. (2007) reported that *S. thermophilus* was resistant...
to cycloserine, kanamycin, metronidazole, nalidixic acid, neomycin, paromomycin, polymyxin B, spectinomycin, and streptomycin (MIC\textsubscript{90} ranging from 64 to 500 µg per mL). It was found highly resistant to aztreonam having a MIC\textsubscript{90} of 1000 µg per mL.

Antibiotic resistance of 39 strains of \textit{S. bovis} representing the microflora of a typical Italian dairy product was found. It displayed high MIC values for tetracycline and the presence of tet(M) was detected in these strains. This poses a potential risk of horizontal transfer of antibiotic-resistance genes among foodborne commensal bacteria (Devirgiliis \textit{et al.}, 2010).

\textbf{Bacillus}

\textit{Bacillus} strains have been increasingly proposed for prophylactic and therapeutic use against several gastro-intestinal diseases (Sorokulova \textit{et al.}, 2008). Reports suggest higher MIC for \textit{Bacillus} strains. In the study of Luna \textit{et al.} (2007), all \textit{B. anthracis} isolates (18) were found resistant to trimethoprim/sulfamethoxazole. Only \textit{B. thuringenesis} (19) was resistant to β-lactams, 3 of 42 isolate of \textit{B. cereus}, 1 of 5 isolates of \textit{B. mycoides} and all species of \textit{B. pseudomycoides} (6 isolates) were resistant to clindamycin. Of 7 erythromycin resistant/intermediate \textit{B. cereus} species, 3 were clindamycin resistant and 1 was both clarithromycin and clindamycin resistant. Vancomycin-resistant \textit{B. cereus} was isolated from respiratory samples from patients in a paediatric intensive care unit of a hospital Kalpoe \textit{et al.} (2008). \textit{B. licheniformis} strain was reported to be resistant to chloramphenicol and clindamycin (Sorokulova \textit{et al.}, 2008).

Presence of mobile plasmid-encoded tetracycline resistance in the \textit{B. cereus} group was mentioned in the EFSA opinion on QPS (European Food Safety Authority-EFSA, 2007). \textit{B. brevis} and \textit{B. firmus} intended to be used as biomass for animal feed were inappropriate for QPS (European Food Safety Authority-EFSA, 2008).

\textbf{Lactococcus}

Some potential risks are involved regarding the use of fermented foods that could act as potential vehicles for the spread of antibiotic resistance to consumers through the food chain. Tetracycline and erythromycin-resistance genes were found among the strains of \textit{Lc. lactis}, representing the fermenting microflora of typical Italian traditional cheese Mozzarella di Bufala Campana. High MIC values for tetracycline were found for 26 strains while 17 strains showed high MIC values for both tetracycline and erythromycin (Devirgiliis \textit{et al.}, 2010).

\textbf{Safety of probiotic foods}

\textit{Lactobacillus}, \textit{Bifidobacterium}, \textit{Pediococcus}, and \textit{Lactococcus} have long history of use in food and extensively been used as probiotics (Shah, 2007). It is estimated that per capita consumption of fermented milk in Europe is 22 kg; this amounts to approximately 8.5 billion kg per year, a total of 8.5 x 10\textsuperscript{20} LAB (assuming 10\textsuperscript{8} cfu per g), and 3400 tones of LAB cells (assuming each cell weighs 4 x 10\textsuperscript{-12} g) (Shah, 2010). US sales of probiotics were estimated to be worth $764 million in 2005 and were projected to be worth $1.1 billion in 2010. Sales of probiotics used in the manufacture of food supplements were projected to reach at $291.4 million in 2010, and food applications are expected to dominate the market, with sales estimated at $700 million in 2010 which include yogurts, kefir, and cultured drinks as major categories (Vanderhoof \textit{et al.}, 2008).

The most common microorganisms used in fermented products belong to the genera \textit{Lactococcus}, \textit{Leuconostoc}, \textit{Pediococcus}, and \textit{Lactobacillus}. Lactobacilli and bifidobacteria are important indigenous microbiota of man and animals, rarely being implicated as cause of infection with quite few exceptions and generally recognized as safe (GRAS). However \textit{B. dentium}, a causative agent of dental caries, was found to be pathogenic. Similarly, \textit{B. animalis} naturally colonizes animal habitats, so its use in humans appears to be inappropriate because the criteria for a probiotic product consumed by humans must contain bacteria from human origin (D’Aimmo \textit{et al.}, 2007).

Based on safety records, microorganisms can be placed in 3 groups: safe strains (\textit{Lactococcus}, \textit{Leuconostoc}, \textit{Pediococcus}, \textit{Lactobacillus}, \textit{Oenococcus}, \textit{S. thermophilus}, \textit{Bifidobacterium}, \textit{Carnobacterium}, \textit{E. saccharolyticus}, and \textit{E. faecium}), doubtful strains (\textit{Enterococcus}, \textit{L. rhamnosus}, \textit{L. catenaforme}, \textit{Vagococcus}, and \textit{B. dentium}) and risky strains (\textit{Peptostreptococcus}, and \textit{Streptococcus}) (Mogensen, 2003). There are 3 theoretical concerns regarding the safety of probiotic organisms: (1) the occurrence of disease, such as bacteremia or endocarditis; (2) toxic or metabolic effects on the gastrointestinal tract; and (3) the transfer of antibiotic resistance in the gastrointestinal flora (Snydman, 2008).

According to Food and Agriculture Organisation (FAO)/WHO guidelines for the evaluation of
probiotics in food (2002), it is suggested that probiotic organisms may theoretically be responsible for side-effects including systemic infections, deleterious metabolic activities, excessive immune stimulation in susceptible individuals and gene transfer. Regarding the safety assurance of probiotic organisms in food, FAO/WHO guidelines (2002) suggest testing probiotic strains for antibiotic resistance patterns, certain metabolic (e.g., D-lactate production, bile salt deconjugation) and hemolytic potential, toxin production, side-effects, and epidemiological surveillance of adverse incidents during human studies and infectivity deficit in immunocompromised animals.

**Animal studies**

The safety concerning the use of these bacteria has not been doubted for many years. However, some of the members of genera *Lactobacillus, Leuconostoc, Pediococcus, Enterococcus,* and *Bifidobacterium* have been frequently reported to be the cause of various infections in patients with clinical conditions such as endocarditis and bloodstream infections (Gasser, 1994). There are many sources of exposure to these bacteria including probiotic preparations, fermented food products as well as the host’s own microflora (Borriello et al., 2003). Since these organisms can adhere to epithelial lining and can survive gastric conditions, they may pose risks of translocation. They can translocate from the gastrointestinal lining to extraintestinal sites. They can enter regional lymph nodes, spleen, liver, blood vessels, and other tissues (Shou et al., 1994) causing systemic infections, bacteremia, septicemia and multiple organ failure (Berg, 1992; Liong, 2008).

Indigenous microorganisms are not normally found in mesenteric lymph nodes, spleen, liver, or blood of healthy subjects. They are eliminated by the host’s immune system as they attempt to translocate across the mucosal epithelium. Thus translocation of probiotic organism is not detected in most of the studies, in which probiotic organisms are administered even at high doses to healthy subjects (Liong, 2008). Lara-Villoslada et al. (2009) found that the strain *L. fermentum* CECT5716 orally administrated to Balb/c mice was non-pathogenic for mice even in doses 10,000 times higher (expressed per kg of body weight) than those normally consumed by humans.

Bacterial translocation does not occur commonly in healthy specific pathogen-free animals but it can be found for a long duration in germ-free mice (Ishibashi et al., 2001). Tanslocation was observed in sterile born mice; however, lactobacilli did not cause any harm and the organisms cleared in 2 to 3 weeks (Mogensen, 2003). *L. delbrueckii* ssp. *bulgaricus, L. rhamnosus,* and *B. lactis* did not translocate. Lara-Villoslada et al. (2007) carried out safety assessment of two probiotic strains including *L. coryniformis* CECT5711 and *L. gasseri* CECT5714 using 20 Balb/c mice which were orally treated with *L. coryniformis* CECT5711 or *L. gasseri* CECT5714 for 30 days and reported no treatment-associated bacterial translocation as these organisms were not present in liver or spleen. In another study, *L. fermentum* CECT5716, a probiotic strain isolated from human milk, was orally administered for 28 days to half of 40 Balb/c mice with a dose of $10^{10}$ colony forming units (cfu) per mouse per day and observed no bacteremia and no treatment-associated bacterial translocation to liver or spleen (Lara-Villoslada et al., 2009). Liong and Shah (2006; 2007) administered *L. casei* and *B. infantis* to 24 rats and no probiotics were detected in the spleen, liver, and kidney suggesting that the organisms were not translocated to these organs. (Tompkins et al., 2008) reported absence of both strains in the liver, kidneys, spleen or heart after 28-days repeated high-dose oral treatment of *E. faecium* R0026, and *Bacillus subtilis* R0179 used in Asian probiotic products, to 30 Sprague-Dawly albino rats.

Intestinal microflora of a subject also plays an important role in the prevention of probiotic translocation to internal organs. In a recent study by Gronbach et al. (2010), it was reported that if both intestinal microbiota and adaptive immunity are defective, translocation across the intestinal epithelium and dissemination of probiotic bacteria such as *E. coli* Nissle could occur with potentially severe adverse effects. Although translocation of probiotic bacteria to internal organs of immunodeficient mice was observed in the study of Wagner et al. (1997), there was no evidence of increased inflammation or other pathologic findings in tissue sections from mice. Zhou et al. (2000) administered *L. acidophilus, B. lactis,* and *L. rhamnosus* to 78 mice at 3 levels including $5 \times 10^7$, $5 \times 10^8$, $5 \times 10^9$ cfu per day and found that the organisms were safe, and no adverse effects were observed.

Animal model could be useful in evaluating the safety of new probiotics in immunocompromised hosts (Borriello et al., 2003). In most of experiments performed in mice, translocation of bacteria is usually observed in immuno-compromised subjects only but the response may vary with age of the animal. Wagner et al. (1997) suggested that the use of probiotic is likely to be safe for immunocompetent and immunodeficient adults, but they should be tested for safety in immunodeficient neonates.
In vitro and in vivo assessments of the safety of two species of Bacillus, including B. subtilis, and B. indicus as a food probiotic were carried out by Hong et al. (2008). The Natto strain of B. subtilis invaded and lysed cells but neither species was able to adhere significantly to any cell line. The Natto strain formed biofilms and none of strains produced any of the known Bacillus enterotoxins. Only B. indicus carried resistance to clindamycin at higher MIC than EFSA breakpoints. In vivo assessments of acute and chronic dosing in guinea pigs and rabbits, no toxicity was observed in animals under these conditions. The authors reported that B. indicus and B. subtilis were safe for oral use but further study is required regarding the transmissibility of clindamycin resistance of B. indicus.

The safety assessment of two Bacillus strains including B. subtilis, and B. licheniformis incorporated into a popular East European probiotic product was carried out. Both were non-hemolytic and did not produce Hbl or Nhe enterotoxins. Similarly, no bceT and cytK toxin genes were found. Study of acute toxicity in BALB/c mice demonstrated no treatment-related deaths. The oral LD_{50} for both strains was more than 2 \times 10^{11} cfu per g. Chronic toxicity studies showed no signs of toxicity or histological changes in either organs or tissues of experimental animals. B. subtilis strain was sensitive to all antibiotics listed by the EFSA but B. licheniformis strain was resistant to chloramphenicol and clindamycin that enclosed safe for oral use but further study is required regarding the transmissibility of clindamycin resistance of B. indicus.

Clinical cases

Documented correlations between systemic infections and probiotic consumptions are few and all occurred in patients with underlying medical conditions (Food and Agricultural Organization of the United Nations/ World Health Organization-FAO/WHO, 2002; Bernardeau et al., 2008). Many of the probiotic organisms have a safe history in patients receiving nutritional support, although some probiotic products have shown to increase the risk of complications in specific patient groups (Whelan et al., 2010).

Table 3. Clinical cases in which lactic acid bacteria or bifidobacteria have been isolated (Adapted from Mogensen et al., 2002)

<table>
<thead>
<tr>
<th>Clinical outcome</th>
<th>Endocarditis</th>
<th>Bacteremia</th>
<th>Other infection</th>
<th>Total</th>
</tr>
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<tbody>
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<td>8</td>
<td>19</td>
<td>34</td>
</tr>
<tr>
<td>L. acidophillus</td>
<td>3</td>
<td>3</td>
<td>2</td>
<td>8</td>
</tr>
<tr>
<td>L. casei</td>
<td>12</td>
<td>-</td>
<td>-</td>
<td>12</td>
</tr>
<tr>
<td>L. plantarum</td>
<td>11</td>
<td>2</td>
<td>1</td>
<td>14</td>
</tr>
<tr>
<td>L. rhamnosus</td>
<td>19</td>
<td>5</td>
<td>3</td>
<td>27</td>
</tr>
<tr>
<td>Bifidobacterium</td>
<td>-</td>
<td>9</td>
<td>-</td>
<td>9</td>
</tr>
<tr>
<td>Leuconostoc</td>
<td>2</td>
<td>23</td>
<td>8</td>
<td>33</td>
</tr>
<tr>
<td>Pediococcus</td>
<td>-</td>
<td>11</td>
<td>7</td>
<td>18</td>
</tr>
<tr>
<td>Total</td>
<td>54</td>
<td>61</td>
<td>40</td>
<td>155</td>
</tr>
</tbody>
</table>

Only about 180 cases of sepsicemia in humans involving LAB have been reported. In only 1 of

International Food Research Journal 18(3): 837-853
these cases, the identified LAB was identical with a commercially available dairy strain. *E. faecium* and *E. faecalis* are more frequently involved in clinical infection. In most cases of infection, people were reported to be infected by their own flora, however, in a few cases consumption of probiotic organisms was a potential source. About 30 cases of fungaemia have been reported in patients treated with *Saccharomyces boulardii* (Gasser, 1994), and 2 cases of infection were with food-borne *L. rhamnosus* (Mackay et al., 1999). In another report, 62 patients became colonized with *B. cereus* including 2 with non-fatal *Bacillus* sepsis and a death due to pneumoiae associated with the organism (Bryce et al., 1993).

Saxelin et al. (1996) studied the prevalence of bacteremia caused by *Lactobacillus* species in Southern Finland and compared the characteristics of the blood culture isolates with probiotic dairy strains. *Lactobacillus* was identified in eight of 3317 blood culture isolates; however, there was no isolate from dairy strain. In a 74-year-old woman with several years history of hypertension and non-insulin dependant diabetes mellitus, liver abscess was reported due to *L. rhamnosus* strain indistinguishable from *L. rhamnosus* strain GG (Rautio et al., 1999).

In a study by Kalliomäki et al. (2001), *L. rhamnosus* GG was given to 132 women who were at high risk of their babies developing atopic dermatitis. There was no report of adverse effects in mothers indicating that the probiotic organism was safe. Reports by Salminen et al. (2002) suggest that *L. rhamnosus* GG has been used widely in Finland since late 1980s and despite the long term use of this probiotic organism, there has been only few cases of bacteremia (0.05 cases per 100 000 cases).

Whelan and Myers (2010) reviewed of total of 1966 articles, of which they found 72 to fulfill the inclusion criteria. There were 20 case reports of adverse events in 32 patients, all of which were infections due to *L. rhamnosus* GG or *Saccharomyces boulardii*. The risk factors included central venous catheters and disorders associated with increased bacterial translocation. There were 52 articles reporting 53 trials in which 4131 patients received probiotic organisms. Most trials showed either no effect or a positive effect on outcomes related to safety (e.g., mortality and infections). Only 3 trials showed increased complications, which were largely non-infectious in nature and in specific patient groups (e.g., transplant and pancreatitis).

Cannon et al. (2005) reviewed 241 clinical cases of *Lactobacillus* infections and found 129 cases of bacteremia and 73 cases of endocarditis. *L. casei* and *L. rhamnosus* were most common species and the overall mortality was reported nearly 30%. Patients of all ages and both gender were affected. The main underlying conditions were recognized as cancer, diabetes, transplantation particularly of liver, abscesses, and hypertension. Husni et al. (1997) reviewed 45 cases of *Lactobacillus* infections occurring over 15 years and the organisms causing infections were characterized. The common underlying conditions were cancer (40%), recent surgery (38%), and diabetes mellitus (27%). One in 39 deaths was attributed to *Lactobacillus* bacteremia. Cannon et al. (2005) recognized a very small percentage (1.7%) of cases associated with heavy dairy consumption, where 3 cases were associated with endocarditis and 1 with a liver abscess. A case of aortic valve endocarditis caused by *L. casei* in a 53-year-old immunocompetent patient with past history of rheumatic fever was reported by Zé-Zé et al. (2004). Noticeably clinical symptoms appeared after a dental extraction and the patient’s diet included several tubs of yogurts per day. Presterl et al. (2001) reported a young man having diet comprising large quantities of probiotic yogurt developed endocarditis and septic arthritis caused by *L. rhamnosus*. However the contradictory findings were reported by Wallet et al. (2002), where a case of endocarditis due to *L. casei* subsp. *rhamnosus* was found in 73-year-old man without previous history of dental manipulation or daily yogurt intake. In relation to a consumption of about 20 million tons of fermented milk annually, the above numbers are negligible (Mogensen, 2003). There is no foundation for safety concern in relation to probiotic dairy products on the market today. Probiotic organisms are generally considered safe. As evidenced by epidemiologic studies, bacteremia or sepsis from lactobacilli is extremely rare. Numerous probiotic organisms have a long history of safe use and no health concerns have been observed. A long history of safe use is still the most credible safety test.

**Conclusions**

Selective pressure of using antibiotic in both human and animal treatment, and dissemination of antibiotic resistance bacteria has the possibility to aggravate acquisition and spread of resistant genes. In this context, probiotic organisms are considered to pool the resistant genes and transfer these to pathogenic bacteria. In order to eliminate this possibility, MIC of the most relevant antimicrobials for each strain used as a probiotic organism, food or feed additives could be determined using protocols given by EFSA and on firm genetic grounds. Several studies regarding
the antibiotic susceptibilities of LAB, bifidobacteria have been reviewed but only few have determined the genetic basis of these resistances. Majority of resistance found in the species of Lactobacillus, Bifidobacterium, Enterococcus, Streptococcus and Bacillus were of intrinsic type. Resistances to tetracycline, vancomycin and erythromycin were frequent in these species and some showed to harbour genes tet(W), tet(M), van(A) and erm(B) mostly on chromosome with only few on plasmid or transposon. Intrinsic resistance, and resistance due to mutation of chromosomal genes present a low risk of horizontal dissemination, and such strains should be acceptable for food consumption. However, acquired resistance mediated by added genes may present a risk for public health. Starter culture bacteria in dairy products do not appear to represent an important source for the spread of genes encoding resistance to antimicrobial agents. However antibiotic resistance profiles of novel strains used as starters or probiotics in dairy products must be checked for fermented dairy products. In case of Enterococcus strains, resistance genes van(A), tet(L), and tet(M) were often detected and 2 reports have found enterococci to transfer tet(M) to E. faecalis or Listeria strains and van(A) to a commercial strain L. acidophilus.

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