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Antibiotic Resistance Profile of Lactic Acid Bacteria and Their Implications in Food Chain

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Abstract: The food chain has been recognized as one of the key routes of transmission of antibiotic resistance from animal to human bacterial populations. The increasing numbers of reports on the presence of antibiotic resistance in food grade bacteria are indicative of an important public health problem globally. There is an urgent need to limit the spread of resistance genes within food grade bacteria, since these could be transferred to opportunistic and pathogenic bacteria. Several initiatives have been recently launched by various organizations worldwide to deal with the bio-safety concerns of starter cultures and probiotic microorganisms. Nevertheless, the prevention and control strategies will require the application of epidemiological and behavioural approach at the national level, too.

Key words: Probiotics · Antibiotic resistance · Biosafety · Fermented dairy products · Food chain

INTRODUCTION

Antibiotics are manufactured at an estimated scale of about 100,000 tons annually worldwide and their use had a profound impact on the life of bacteria on earth [1]. In developing countries, their use in food and agriculture sector and in livestock industries are limited and applied only for therapeutic measures. In many countries antibiotics are available without prescription also and are mostly used inadequately in sub-therapeutic doses [2, 3]. There are no governmental regulations on the use of antibiotic in agriculture [4, 5] and thus, this uncontrolled antibiotic use finally contaminates the dairy food chain.

Antibiotic resistance or drug resistance can be defined as the ability of bacteria and other microorganisms to withstand an antibiotic to which they were once sensitive (and were once stalled or killed outright). More strains of pathogens have become antibiotic resistant and some have become resistant to many antibiotics and chemotherapeutic agents. This phenomenon is called multidrug resistance, which makes the antibiotic resistant bacteria a well known worldwide risk on human health [4]. This has been recently defined as a shadow epidemic [6] and especially milk represents a source where resistant bacteria can enter the human food chain [7].

According to the European Commission [8], it has been estimated that somewhere from one to ten million tons of antibiotics have been released into the biosphere over the last 60 years. This has lead to a very strong selective pressure for the appearance of resistant bacterial strains; among which much concern has been about pathogenic bacteria. Consequences of antibiotic resistance include increased morbidity and mortality, enhanced stay in hospitals leading to increased duration and cost of treatments [9]. Irrational use coupled with lack of development of new antibiotics has made the situation worrisome.

Now a days increased attention is given to safety of bacteria applied in food. Lactic acid bacteria (LAB) are natural and profitable inhabitants in many environments including vegetables, soil, gastrointestinal (GI) tract etc. LAB strains with resistance to antibiotics would not be detrimental to the wellbeing of humans or animals. However, there is some concern that antibiotic resistance in LAB could then be transferred to pathogenic bacterial species, complicating the treatment of a disease or infection and lead to the spread of antibiotic-resistant strain [10]. Scott [11] stated that gene transfer occurs widely *in vivo* between GI tract bacteria and pathogenic bacteria, as identical resistance genes are present in diverse bacterial species from different hosts. Breakpoints

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used for antibiotic susceptibility profiling are harmonized in Europe by the EUCAST but unfortunately no harmonized guidelines for testing non-*enterococcus* LAB are available and therefore results are not well comparable [8, 12].

History of Development of Antibiotic Resistance: Resistant bacteria have always been around and existed long before humans began using antibiotics therapeutically. Right after the beginning of use of penicillin, some *Staphylococcus* strains were identified as resistant to the antibiotic and today 80% of *Staphylococcus* strains do not respond to penicillin [6]. In the 1940s and early 1950s, streptomycin, chloramphenicol and tetracycline were discovered. By 1953, a strain of *Shigella* was found that resisted these antibiotics and sulfanilamides. During 1940-50s, a single antibiotic, such as streptomycin, was effective against causative agent of tuberculosis is now no longer able to cure the disease anymore. Additionally, multi-drug resistant tuberculosis strains have arisen and thus, tuberculosis is the leading cause of death by infectious disease in the world. The resistant strains of *Gonorrhea* arose by the 1970s and from1990s, the development of true superbugs, bacteria that resist all known antibiotics was scrutinized. One antibiotic of last resort is vancomycin, a powerful antibiotic that attacks bacteria on many fronts, but now there are *enterococci* strains that resist vancomycin [13].

Antibiotic Resistance in the Food Chain: There is a close association between the quantities of antimicrobials being used and the rate of development of resistance to these substances. Hence, the misuse of antibiotics in human medicine is believed to be the principal cause of the antibiotic resistance problem [14]. Another aspect is the selection of resistant bacteria in the food chain due to the heavy utilization of antimicrobial agents in animal husbandry [15, 16]. Several reports state that antibiotic residues have been found in milk [17]. Although clinical organisms have been considered primary culprits contributing to emergence and transfer of resistance, the role of food chain commensal organisms such as LAB has not received due attention and thus, studies concerning the antibiotic resistance in normal bacterial flora is limited. The food chain can be considered as the main route of transmission of antibiotic resistant bacteria between the animal and human population [18-20]. Moreover, fermented dairy products and fermented meats that are not heat-treated before consumption provide a vehicle for antibiotic resistant bacteria with a direct link between the animal indigenous microflora and the human GI tract [21]. Clinical investigations have documented persistence of antibiotic resistant strains in the human gut even in the absence of selective pressure, indicating that drug exposure induces long-term alterations within complex microbial communities [22].

Antimicrobial susceptibility testing may perhaps be performed using different phenotypic methods. In that, agar dilution and broth micro-dilution are the regular methods as per CLSI (Clinical and Laboratory Standards Institute, formerly NCCLS, National Committee on Clinical Laboratory Standards). Other widely used methods include the agar gradient method and commercial methods, such as E-test, which consists of a predefined gradient of antibiotic concentrations on a plastic strip (AbBiomerieux, Sweden). In addition to the determination of phenotypic antibiotic resistance, the genotypic detection of particular genes causing resistance is also essential. These genotypic methods include different PCR- based methods, southern hybridization, plasmid profiling and microarray [23, 24]. The situation is apparent when the phenotypic and genotypic resistance patterns are in agreement, however, a phenotypically resistant bacterium strain may be genotypically "susceptible". This is usually due to the fact that appropriate genes are not included in the test patterns, or there exist unknown resistance genes. Tetracycline, for example, has more than 40 different genes conferring antibiotic resistance discovered and the number of tetracycline resistance genes continues to increase [25].

LAB and Antibiotic Resistance: LAB are a broad group of Gram-positive, non-sporing rods and cocci, usually non-motile that ferment carbohydrates and form lactic acid as the major end-product [10]. *Bifidobacteria* and LAB including *Lactococci, Lactobacillus*, *Enterococcus* and *Pediococcus* are a significant part of the food chain and widespread in human, animal and plant microflora. They have a role as commensals on mucosal surfaces and skin and inhabit the digestive tract of many animal species, but their prevalence and distribution vary according to the animal species [26-28]. LAB often harbour plasmids of different sizes and some antibiotic resistance determinants located on plasmids have been reported to occur in *Lc. lactis* and various *Lactobacillus* and *Enterococcus* species [29]. Among the LAB, antibiotic resistance of the enterococci has been subject to intense study particularly because strains of these bacteria cause numerous and serious infections in humans [30, 31]. In contrast, fewer

physiological and molecular data are available on the antibiotic resistances of lactobacilli present in fermented foods.

There are three types of resistance observed in LAB: intrinsic or innate, acquired and mutational. The knowledge of intrinsic, chromosomally coded resistance of LAB to common antibiotics is necessary to recognise acquired resistance traits [21]. Bacteria that do not begin life resistant to a certain antibiotic can acquire that resistance. Mutations, which may cause genetic changes in multiple regions of the genome, play only a minor role in the development of resistance [32, 33]. In the case of vertical evolution and inherent resistance, mutations occur on chromosomes and are then selected for an environment where resistance increases fitness. In the case of horizontal evolution, genes pass from a resistant strain to a non-resistant strain, conferring resistance on the latter. According to European Commission [8], strains carrying the acquired resistance due to acquisition of exogenous resistance genes are unacceptable for use as animal feed additives.

Mechanisms of Antibiotic Resistance: A single bacterial strain may possess several types of resistance mechanisms. The resistance mechanism categorized as biochemical and genetic types. Among these, which mechanism prevails in the specific bacterial strain depends on the nature of the antibiotic, its target site, the bacterial species itself and whether it is related by a plasmid or by a chromosomal mutation.

- Biochemical aspect: Although the manner of acquisition of resistance may vary among bacterial species, resistance is created by only a few mechanisms: (*i*) Antibiotic inactivation - direct inactivation of the active antibiotic molecule (*ii*) Target modification - alteration of the sensitivity to the antibiotic by modification of the target (*iii*) Efflux pumps and outer membrane (OM) permeability changes - reduction of the concentration of drug without modification of the compound itself, or (*iv*) Target bypass - some bacteria become refractory to specific antibiotics by bypassing the inactivation of a given enzyme [16, 21]. Most of these mechanisms have been observed and studied in various bacteria, however, there have not been specific studies dealing with these mechanisms in LAB or bifidobacteria.
- Genetic aspect*:* The naturally occurring mechanism of antibiotic resistance involves mutations or horizontal transfer of resistant genes among the bacteria [16]. The evolution of antibiotic resistance in microbial communities is enhanced by horizontal transfer of resistance genes over species and genus borders by (i) conjugative plasmids, (ii) transposons, (iii) the possession of integrons and insertion elements; and (iv) lytic and temperate bacteriophages [19, 34]. In general, two of the most commonly observed resistance genes in LAB found so far are *tet*(M)-for tetracycline resistance and *erm*(B)-for erythromycin, followed with *cat* genes coding for chloramphenicol resistance [29, 35, 36].
- *Conjugative Plasmids*: Conjugal gene transfer between enterobacteria was discovered due to detection of high level multiple antibiotic resistance in *Shigella* and *E. coli* isolates during a dysentery epidemic in Japan. Indeed, plasmids are common in enterococci, lactococci, leuconostoc, pediococci and in few strains of *S. thermophilus*, lactobacilli and bifidobacteria [19, 37]. A well characterized broad host range conjugative plasmid is pAMβ1 which was isolated from *E. faecalis* and found to carry a constitutive MLS resistance (macrolides, lincosamides and streptogramin B). Many lactobacilli in contrast to lactococci are resistant to conjugative transfer of the broad host range resistance plasmid pAMβ1. Another broad host range plasmid pIP501, originally isolated from *Stre. agalactiae* can be conjugated into *Streptococcus, Staphylococcus, Clostridium*, *Listeria* and *Pediococcus* species and it carries a MLS resistance gene along with a region responsible for conjugative transfer and a gene for a chloramphenicol acetyl transferase [38].

Conjugative plasmids have not been revealed in *Bifidobacterium* [39]. A similar situation is evident in *Leuconostoc* and *Pediococcus* which, however, can accept broad host range antibiotic resistance plasmids like pAMβ1, pIP501 and pVA797:Tn*917* by high-frequency-conjugation from *Lactococcus* harbouring these plasmids [40].

- *Conjugative transposons*: Conjugative transposons, broad and narrow host range have been found in enterococci, lactococci and streptococci and they are considered as a main type of vehicle regarding

antibiotic resistance transport in Gram positive bacteria [21]. They have been discovered in *E. faecalis* (Tn*916*, Tn*918*, Tn*920*, Tn*925*, Tn*2702*), *E. faecium* (Tn*5233), S. pyogenes* (Tn*3701)* and *Lc. lactis* (Tn*5276*, Tn*5301*). In enterococci and streptococci, they determine resistances to tetracycline (*tetM*), erythromycin (*ermAM, erm*), chloramphenicol (*cat*) and kanamycin (*aphA3*). In lactococci, they code for nisin (*nis*) production and sucrose fermentation (*sac*). They vary in size between 16 to 70 kb and may mobilize plasmids or chromosomal genes in one or multiple copies/ inserts [37].

Within Tn*916*/Tn*1545* family, the most remarkable observation is the extreme host range including the genera: *Acetobacterium, Acholeplasma, Alcaligenes, Bacillus, Butyrivibrio, Citrobater, Clostridium, Enterococcus, Escherichia*, *Eubacterium, Fusobacterium, Haemophilus, Lactobacillus, Lactococcus, Leuconostoc*, *Listeria, Mycoplasma, Neisseria, Peptostreptococcus, Staphylococcus, Streptococcus, Thermus* and *Veillonella* and probably more which have not yet been investigated [41]. Non conjugative transposons Tn*1546* which carries the *vanA* gene cluster responsible for vancomycin resistance in enterococci found to be mobilized by conjugative plasmids [42].

- *Integrons and Insertion sequences* (ISs): IS are small segments of DNA flanked by short repeated sequences required for transposition and encoding only few functions involved in their own mobility [43]. Like transposons, IS elements have been found on chromosomes, on plasmids or on both, but their horizontal transfer occurs only when they are associated with conjugative elements. The genome sequencing of the vancomycin resistant V583 strain of *E. faecalis* has shown presence of 38 IS which, together with other mobile elements and exogenously acquired DNA, represent more than 25% of the entire genome [44].
- *Lytic and temperate bacteriophages*: Transfer of antibiotic resistance genes within LAB by bacteriophages and prophages seems theoretically possible, but has not been studied to any extent in LAB [37]. Moreover, the host range of such transfers is limited to closely related strains within one species.

Antibiotic Resistances in the Food-associated LAB: An overview of antibiotic resistances reported in the food-associated LAB is compiled in Table 2. Most of the studies represent erythromycin, tetracycline, chloramphenicol and vancomycin resistance of lactobacilli from different dairy products such as cheese [7, 19, 45-47], yoghurt [48] and fermented beverages [49]. Resistance with neomycin and polymyxin B of the yoghurt cultures was documented by Sozzi and Smiley [50] in one of the old studies. Apart from that, most of these studies focused on evaluating the antibiotic resistance of different LAB strains while in few [46] it is limited to different species of the single LAB strain.

Few studies indicate that antibiotic resistant LAB are widespread among traditional Chinese fermented foods and their resistance incidences depended on the raw material and manufacturing area of the foods. In an experiment carried out by Pan *et al*. [51], it was observed that resistance incidences of fermented sausages were much higher than that of fermented vegetables. Out of 202 LAB isolates, in fourteen strains multi-resistance was observed by the detection of tetM and ermB genes on both plasmids as well as on chromosomes. Recently, Zhou *et al.* [52] for first time detected the aph(3')-IIIa and ant(6) genes along with tet(M) responsible for antibiotic resistance in the isolated yoghurt cultures through PCR; however, horizontal transferred to other species were not analysed among the isolates. In one of studies *tet* genes were identified from 12 strains (out of 73) of lactobacilli including *L. kefiri* NWL78 isolated from a probiotic yogurt [53]. In the same study, erm(B) gene was also detected in some of the isolates and in filter mating experiments, the erm(B) gene from *L. fermentum* NWL24 and *L. salivarius* NWL33 and tet(M) gene from *L. plantarum* NWL22 and *L. brevis* NWL59 were successfully transferred to *Enterococcus faecalis* 181. Although probiotic products and starter strains rarely had acquired antibiotic resistance such results depict the attention for a strict monitoring and regulation. In this context, microbiological break points for categorizing LAB and some non-LAB as resistant are defined by European Commission [54] as shown in Table 3.

The transfer of vancomycin resistance (*vanA*) from enterococci to a commercial *L. acidophilus* strain was observed *in vitro* and *in vivo* in mice [55], however there are no such other reported studies. It has been mentioned that resistance to QAC benzalkonium chloride (BC), widely used as disinfectant in medical and food

Table 1: Intrinsic antibiotic resistance profile of LAB and Bifidobacterium spp. (modified from Teuber *et al.*[37])

Type of bacteria	Intrinsic Antibiotic Susceptibility	Intrinsic Antibiotic Resistance
Bifidobacterium	Ampicillin, penicillin G, bacitracin, cephalosporin, chloramphenicol, erythromycin, clindamycin, nitrofurantoin, tetracycline.	Vancomycin, gentamycin, fusidic acid, streptomycin, polymyxin B, trimethoprim, aminoglycosides, colistin, metronidazol
Enterococci	erythromycin, streptomycin, gentamycin, penicillin G, tetracycline, chloramphenicol	Kanamycin
Lactococcus lactis	Amikacin, ampicillin, 1st generation cephalosporine, chloramphenicol,erythromycin, gentamicin, penicillin, imipenem, oxacillin, sulfonamide, tetracycline, vancomycin	Colistin, fosfomycin, pipemidic acid and rifamycin.
Lactobacilli	Chloramphenicol, streptomycin, gentamycin, penicillin G, tetracycline and Erythromycin	Aminoglycosides, fluoroquinolones, glycopeptides and vancomycin

Table 2: Overview of antibiotic resistances in the food-associated LAB.

Foods	Species	Resistance	Detection and location of gene	References
Chinese yoghurts	*S. thermophilus* and *L. delbruekii* ssp. *bulgaricus*	Ampicillin, kanamycin, chloramphenicol, chlortetracycline, tetracyclines, neomycin and gentamycin	*tet* M, *ant* 6, aph 3'-IIIa	Zhou *et al.*[52]
Chinese fermented foods-pickles, sausages	*L. plantarum, L. fermentum, L. helveticus, Ent. faecium*	Tetracycline, erythromycin, chloramphenicol, kanamycin	*tet* M and *erm* B, -plasmid and chromosome; gene *aph* A3, - plasmid, gene *mef* A, -chromosome	Pan *et al.*[51]
Chinese fermented foods	*L. fermentum* NWL24 and *L. salivarius* NWL33; *L. plantarum* NWL22 and *L. brevis* NWL59 *L. brevis* and *L. kefiri*	Erythromycin 11%., tetracycline 17%., gentamycin 65%., ciprofloxacin 85%.	*erm* B, *tet* M, *tet* S	Nawaz *et al.* [53]
Italian fermented products	*L. paracasei* 197 strains.	Tetracycline 22%., erythromycin 6%.,		Comunian *et al.*[46]
Italian Sola cheese made from raw milk	*L. sakei* Rits 9	Tetracycline, erythromycin	*tet* M, - transposon; *tet* L, - plasmid	Ammor *et al.* [45]
Italian dairy product	Lc. lactis, Stre. bovis, Ent. faecalis,	Tetracycline, erythromycin		Devirgiliis *et al.*[47]
Raw milk, starter-free cheese	Lc. Lactis	Tetracycline	*tet* M, on plasmid	Florez *et al.* [12]
Turkish yoghurt	*S. thermophilus*	Vancomycin 65%.		Aslim and Beyatli [48]
Fermented dry sausages	*Lactobacillus* species	Tetracycline gentamicin 79%. penicillin g 64%. kanamycin 79%.		Gevers *et al.* [29]
Indian vegetables and fermented foods	*L. plantarum, L. fermentum, Weissella* spp. *P. parvulus*	Gentamicin, vancomycin, norfloxacin, kanamycin		Patel *et al.* [65]
European probiotic products	*L. acidophilus, L. rhamnosus, L. casei, L. reuteri, L. johnsonnii,L. plantarum, L. delbreukii spp. Bulgaricus*	Tetracycline 26%. Penicillin g 23%. Erythromycin 16%. chloramphenicol 11%.		Temmerman *et al.*[66]
Raw milk soft cheese	*Lc. lactis* strain K214	Streptomycin, tetracycline, chloramphenicol	*Str-tet* S. *-cat*	Perreten *et al.* [7]
Greek cheese	*L. acidophilus* ACA-DC 243	Penicillin		Charteris *et al.*[67]
Yoghurt starter cultures	*S. thermophilus* and *L. delbruekii* ssp. *bulgaricus*	Neomycin, polymyxin B		Sozzi and Smiley [47]
Nigerian fermented foods and beverages	*L. pentosus, L. acidophilus, L. casei, L. brevis, L. plantarum, L. jensenii*	Tetracycline 42.5%. Erythromycin 17.5%. Ampicillin 47.5%. cloxacillin 80%. ; penicillin 77.5%. ;		Olukoya *et al.* [46]

Table 3: Microbiological breakpoints categorizing bacteria as resistant mg L^{-1}. Strains with MICs higher than the breakpoints below are considered as resistant.

	ampicillin	vancomycin	gentamicin**	kanamycin**	streptomycin**	erythromycin	clindamycin	quinupristin + dalfopristin	tetracycline	chloramphenicol
Lactobacillus obligate homofermentative	1	2	16	16	16	1	1	4	4	4
Lactobacillus helveticus	1	2	16	16	16	1	1	4	4	4
Lactobacillus acidophilus group	1	2	16	16	16	1	1	4	4	4
Lactobacillus delbrueckii	1	2	16	16	16	1	1	4	4	4
Lactobacillus obligate heterofermentative	2	n.r.	16	16	64	1	1	4	8	4
Lactobacillus reuteri	2	n.r.	8	16	64	1	1	4	16	4
Lactobacillus fermentum	1	n.r.	16	32	64	1	1	4	8	4
Lactobacillus facultative heterofermentative*	4	n.r.	16	64	64	1	1	4	8	4
Lactobacillus plantarum	2	n.r.	16	64	n.r.	1	1	4	32	8
Lactobacillus rhamnosus	4	n.r.	16	64	32	1	1	4	8	4
Lactobacillus paracasei	2	n.r.	32	64	n.r.	1	1	4	4	4
Bifidobacterium	2	2	64	n.r.	128	0.5	0.25	1	8	4
Enterococcus	4	4	32	512	128	4	4	4	2	8
Pediococcus	4	n.r.	16	64	64	1	1	4	8	4
Leuconostoc	2	n.r.	16	16	64	1	1	4	8	4
Lactococcus lactis	2	4	32	64	64	2	4	4	4	8
Streptococcus thermophilus	2	4	32	64	64	2	2	4	4	4
Bacillus spp	n.r.	4	4	8	8	4	4	4	8	8
Propionibacterium	2	4	64	64	64	0.5	0.25	0.5	2	2
Other Gram +	1	2	4	16	8	0.5	0.25	0.5	2	2

n.r. not required. *I*ncluding *Lactobacillus salivarius*. ** Possible interference of the growth medium (European Commission, [54[).

environments is not frequent in LAB isolated from food and food environments, but resistance may occur after exposure to BC [56]. During study, the BC resistant isolates showed no cross-resistance with other antimicrobial compounds, except for gentamycin and chlorhexidine. The known *qacA*, *qacB*, *qacC/smr*, *qacG* and *qacH* genes found in staphylococci are generally plasmid-borne [41, 57-59]. Presence of the *ebr* gene (identical to *qacC/qacD*) in enterococci has been reported [60].

Recently, Bennedsen *et al.* [61] tested 28 strains of LAB and bifidobacteria for the presence of >250 antimicrobial resistance genes and >400 toxin and virulence factor genes. The results revealed that *L. lactis* CHCC6005 carries *tet*(S) gene on a medium-copy-number plasmid which should be cured before using the strain to suppress spread of antibiotic resistance through consumption of dairy food. Similarly, all three *B. animalis* subsp. *lactis* strains contained *tet*(W), however, transfer of *tet*(W) from this bacterium to other bacteria has never been demonstrated [62]; thus, *tet*(W) is not considered to be transmissible.

Apart from this in a recent study, El-Adawi and El-Deeb [63] investigated the ability of the extra- and intra-cellular extract of LAB to cure plasmid acquiring resistance in certain clinical antibiotic-resistant bacterial isolates including *Pseudomonas aeruginosa, Staphylococcus aureus, Klebsiella pneumoniae* and *Shigella* spp. The LAB extract mediated plasmid curing resulted in the subsequent loss of antibiotic resistance encoded in the plasmids as shown by antibiotic resistance profile of cured strains. These fascinating results reveal new direction in controlling spread of plasmid mediated antibiotic resistance among the pathogens by LAB. At the same time investigations involving such novel studies are required to claim the role of LAB as an anti-plasmid (plasmid borne multiple antibiotic resistance) agents of natural origin.

Preventive Measures: The transfer of antibiotic resistant bacteria from animals into fermented and other foods can be avoided if the raw substrate (milk or meat) is pasteurized or heat-treated [21, 64]. In addition, generation of antibiotic resistant bacteria in food animals and plants has to be minimized by prudent use of antibiotics. To preserve the life saving potential of antibiotics the spread of resistant genes at all levels must be stopped. This includes the ban of antibiotics with clinical application as growth promoters in animal husbandry as recently established politically in the European

Union and Switzerland; antibiotics with cross resistance to compounds used in human medicine (tylosin, virginiamycin) or used as such in human medicine like bacitracin have been banned [20]. Antibiotic resistance traits as selectable markers in genetic modification of LAB for different purposes are presently being replaced, e.g. by metabolic traits to generate food grade vectors. To prevent the undesirable transfer of resistance or conferment of resistance to endogenous bacteria, probiotics should not carry resistance other than that required.

CONCLUSIONS

Antibiotic resistance is present in a various bacterial species and the responsible genes are detectable in strains with resistant phenotypes. The potential transferability of these resistant genes poses a threat to food safety. MIC break points of LAB require standardization and evaluation of the safety of LAB for human consumption must be guided by established criteria, guidelines and regulations and standardized methods for pre-market bio-safety testing and post market surveillance. Action must be taken to slow the rate of evolution and spread of antibiotic resistance genes, in which the biggest single factor is the amount of antibiotics used in human medicine and agriculture. Thus, prevention and control strategies will require the application of epidemiological and behavioural approaches, as well as the research technologies aimed at the basic mechanisms of drug resistance. The role of LAB or their metabolites as a natural curing agent should be encouraged in order to control plasmid mediated gene transfers among the clinical strains as well as in food grade bacteria, too.

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