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Antibiotic Resistance (ABR) in Lactic acid Bacteria (LAB)

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ABSTRACT

LAB are employed as starters in the manufacturing of fermented milk products; they are also found in naturally fermented foods, and a few species are found in the human intestine as probiotic bacteria. LAB has previously been granted GRAS (generally recognized as safe) and QPS (Qualified Presumption of Safety) designation by the US Food and Drug Administration (FDA) and the European Food Safety Authority (EFSA), respectively. However, the recent discovery of antibiotic resistance in LAB, as well as continued exposure to environmental circumstances, may promote LAB as intrinsic or extrinsic storage of antibiotic resistant genes, which might be horizontally transmitted to other bacteria through the food chain. In LAB, the ABR gene is found on chromosome or plasmid DNA and can be transferred to other organisms in the same ecosystem by conjugation or transformation. The risk of transferring the same resistance genes into pathogenic strains, therefore increasing the resistance profile of these bacteria and complicating infection treatment, is the ultimate dilemma of resistance transfer across LAB or non-pathogenic, commensal species.

Key words–Antibiotic, Antibiotic Resistance, Antibiotic residues, Fermented foods, Generally Recognized as Safe

1. INTRODUCTION

Antibiotics are widely used in farm animal feed and the poultry industry to prevent disease and increase animal performance. During the dry period, antibiotics are frequently given to the entire herd to prevent mastitis. Increased usage of antimicrobials in a herd usually means more antibiotics are used, which increases the risk of antibiotic residues in milk and bacterial resistance to antimicrobials. Antimicrobials are widely used in poultry farms to keep chicks from being infected with illnesses, which is a major problem for the industry. Antibiotic resistance has accumulated in the pathogenic microbiome as a result of their widespread and excessive use. LAB have a long history of usage as probiotics and in fermented foods. Despite this, numerous recent studies have found that the LAB acquire ABR (Antibiotic Resistant) genes both intrinsically and extrinsically. The likelihood of spreading these ABR genes to pathogenic as well as beneficial bacteria of the human gut system, as fermented foods are ingested by humans, poses a severe hazard. Antibiotics, antibiotic classification, antibiotic resistance (ABR) among LAB, mechanism transfer of ABR genes, and spread into harmful bacteria are all topics covered in this review.

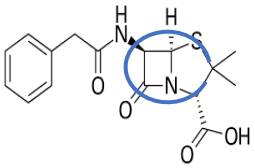
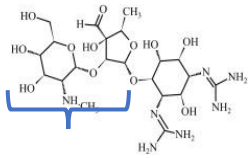
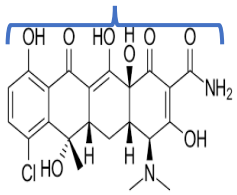
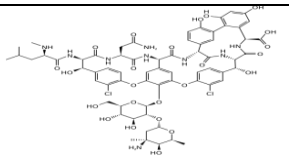
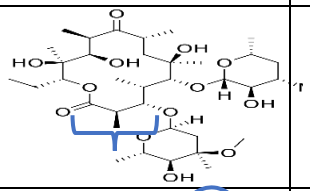
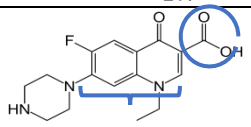
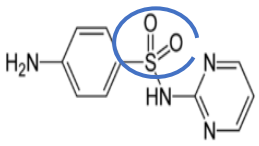
2. LITERATURE REVIEW

2.1 Definition of antibiotic

Antibiotics are "organic substances produced by one microbe that are poisonous to other microbes" (Russel,2004). 'Antimicrobials can also be made entirely or partially synthetically' (Etebu and Arikekpar,2016). Antibiotics are "antimicrobial substances that are natural, synthetic, or semi-synthetic" (Catteau et al., 2018).

2.2 Classification of antibiotics

The most common classification schemes are based on their molecular structures, mode of action and spectrum of activity(Calderon and Sabundayo.,2007).

Group	Chemical structure	Examples	Mode of action
β-lactams	 ($C_{16}H_{19}N_3O_5S$)	Penicillin G Narrow spectrum (Only on G+ve) Amoxicillin ($C_{16}H_{25}N_3O_8S$) Broad spectrum (Both- G+ve& G-ve)	- Inhibits cell wall biosynthesis - Scarlet fever
Aminoglycosides		Streptomycin ($C_{21}H_{39}N_7O_{12}$)	- Inhibits cell wall biosynthesis - *Helicobacter pylori* ulcer
Tetracyclines		**1st genBiosynthesis** Chlortetracycline ($C_{22}H_{23}ClN_2O_8$) **2nd gen- Synthetic** Doxycycline ($C_{24}H_{33}ClN_2O_{10}$) **3rd gen-Total synthesis** Tigecycline ($C_{29}H_{39}N_5O_8$)	- 30s Ribosome Inhibit protein synthesis - Rocky Mountain spotted fever
Glycopeptides		Vancomycin ($C_{66}H_{75}Cl_2N_9O_{24}$)	- Inhibits - cell wall synthesis - MRSA (Methicillin - resistant*S. aureus*) infection
Macrolides		Erythromycin ($C_{38}H_{72}N_2O_{12}$)	- 50s Ribosome Inhibit protein synthesis - Pneumonia
Quinolones		Norfloxacin ($C_{16}H_{18}FN_3O_3$)	- DNA Gyrase - Typhoid
Sulphonamides		Sulfadiazine ($C_{10}H_{10}N_4O_2S$)	- Inhibit - Folic acid synthesis - Travelers diarrhoea

Most antibiotics have antibacterial efficacy that targets a specific characteristic of the bacterial structure or metabolic functions. The following are the mechanisms of antibiotic activity (Madigan and Martinko, 2006; Talaro and Chess, 2008):

• Penicillin G and Amoxicillin are examples of antibiotics that inhibit cell wall production.
• Degradation of cell membrane structure or function (Polymyxin, Daptomycin, etc.).
• Inhibition of nucleic acid structure and function, such as Nalidixic acid and Ciprofloxacin.
• Protein synthesis inhibitors, such as erythromycin and tetracycline.
• Sulphonamides and Trimethoprim, for example, block important metabolic processes.

Antibiotics belonging to the same structural class will have similar efficacy and allergic-potential adverse effects.β-lactams, Aminoglycosides, Tetracyclines, Glycopeptides, Macrolides, Quinolones, and Sulphonamides are some of the most frequent antibiotic

groups based on chemical or molecular structures (Adzitey,2015).

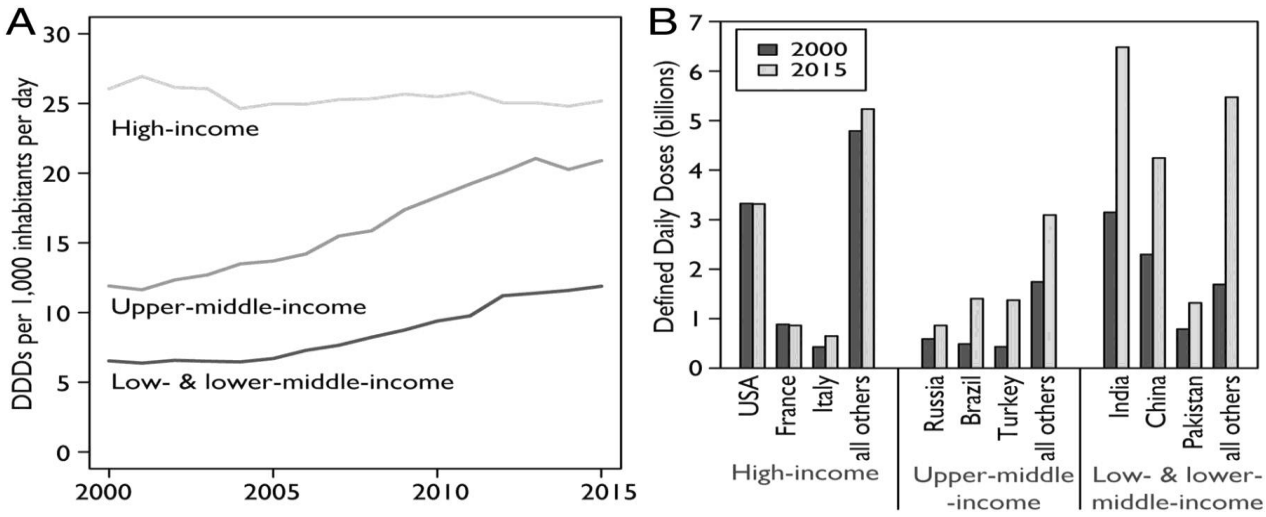
2.3 Antibiotic Consumption Trend Globally and in India (Klein *et al*., 2018)

Trend	Global	India
Consumption (Daily Defined Dose's-DDDs)	Increased 65 % (21.1–34.8 billion)	Increased 103 % (3.2 to 6.5 billion)
Consumption Rate (DDDs/1000inhabitants/day)	Increased 39% (11.3 to 15.7 DDDs)	Increased 63% (8.2 to 13.6 DDDs)

China was the world's producer of antibiotic. Consumption rate of broad-spectrum antibiotic penicillin was 39 % total DDDs in 2015 and it has increased to 36 % between 2000 to 2015 globally.

❑ **Consumption rate and consumption of Antibiotics among countries**

High income Countries	Upper middle-income Countries	Lower middle-income countries
USA	Russia	India
France	Brazil	China
Italy	Turkey	Pakistan
Others (Spain, Greece)	Others (South Africa, Bulgaria)	Others (Vietnam, Egypt)



2.4 Lactic Acid Bacteria (LAB)

Lactobacillus, Streptococcus, Leuconostoc, Enterococcus, and Pediococcus, which are low in G + C with 31–49 percent, belong to the Bacilli class and *the Lactobacillalesorder*, but the *Bifidobacterium* genus, which has a high G + C content (58–61 percent), belongs to the *Actinobacteria* phylum ((Fraqueza,2015).

Lactic Acid Bacteria (LAB) are a taxonomically diverse group of Gram positive, facultative anaerobic, non-spore forming, non-motile, and acid-tolerant cocci, coccobacilli, or rods that appear as single cells, pairs, tetrads, or long chains and have a common metabolism and physiology capable of fermenting sugars primarily into lactic acid (Cisneros and Alquicira,2018).

2.5 Classification of starters LAB

- Based on their optimum development temperature, starters used in the production of fermented milk products are classified as mesophilic or thermophilic. Lactococci, Pediococci, Leucocnostoc, and Lactobacilus brevis are mesophilic starters, while Streptococcus thermophilus, Lactobacillus delbrueckiissp. bulgaricus, and Lactobacilus acidophilus are thermophilic starters. Starters can be homofermenters, heterofermenters, or various fermenters, depending on the kind of fermentation. Lactococci, Streptococcus thermophilus, Lactobacillus delbrueckiissp. bulgaricus, and Lactobacillus acidophilus are homofermenters that

produce only lactic acid; heterofermenters include Leuconostoc, Leuconostoc brevis, Leuconostoc fermentum, and Bifidibacteriumbifidum, which produce lactic acid as well as other acids and/or Miscellaneous fermenters include Propionibacterium freudenreichiissp. shermanii, Kluveromyces, and Torula, which do not generate lactic acid (Robinson and Tamime, 1998).

2.6 Role of LAB

- Lactobacillus spp., Leuconostoc spp., Lactococcus spp., and other LAB strains are the most frequent microbes found in fermented milk products. LAB species can be found in a variety of genera within the Lactobacillaceae family. They are potential microorganisms that have been frequently used in food fermentation around the world because of their well-known GRAS status. They're also known for their fermentative abilities, which help to improve food safety, organoleptic qualities, nutrient enrichment, and health advantages. (Rakhmanovaet al., 2018; Hao et al., 2014; Yantyatiet al., 2014)

- **Role of Lactic acid bacteria in Fermented milk Products**

Function	Changes	Effect
Primary	Lactic acid production	Inhibit undesirable bacteria like *Pseudomonas, Micrococci.*
Secondary	Acid coagulation of milk	Curdling effect on milk
	Flavour production of diacetyl, acetaldehyde by aroma producing LAB	Adds diacetyl flavour to Dahi& acetaldehyde to Yoghurt product.
	Bacteriocins are polypeptides synthesized by LAB, example - Nisin - *Lactococcus lactis ssp. lactis* NIZ02218, Acidophilin from *Lactobacillus acidophilus*	Suppress potential pathogens and spoilage organisms – *S. aureus*, Micrococci & bacterial spores
	Some LAB, example *Lactobacillus helviticus*produce exopolysaccharide	Improves texture by increasing the viscosity of fermented milk products.

2.7 ABR in the laboratory

LAB are inherently resistant to a number of antibiotics, and they may have the potential to develop resistance to other antimicrobials or to spread resistance to pathogens found in the gastrointestinal tracts of both animals and humans. Because fermented milk and meat products are the most common vehicle for antibiotic-resistant bacteria to reach the indigenous flora of the gastrointestinal tract, the food chain can enhance the transmission of antibiotic-resistant bacteria between animals, foods, and humans. Despite this, resistant determinants are transmitted in LAB via the two most prevalent resistant genes, tetracycline [tet(M)] and erythromycin [erm(B)], followed by cat genes coding for chloramphenicol resistance (Preethi et al., 2017)

2.8 Transfer mechanisms of ABR genes

LAB are thought to be resistance gene carriers, with the ability to spread their genes across the food chain, as well as to the environment, via various processes. Antibiotics are used in animals meant for human consumption as growth boosters or pathogen inhibitors. Antibiotics may cause bacteria in the gastrointestinal tract to evolve several methods to counteract the action, including innate and acquired resistance mechanisms (Mathur et al., 2005). The technique used by bacteria differs depending on the antibiotic, the target location, the bacterial species, and/or whether the resistance gene is found on the chromosome or in mobile elements such as plasmids or transposons (Imperial and Ibana,2016)

2.9 Resistance Mechanisms in LAB

The antibiotic-target interaction requires two elements: first, the antibiotic must recognize the target, and second, the antibiotic concentration in the target must be adequate to suppress bacterial growth. Due to an inadequate antibiotic-target interaction, which can be categorized as passive or active, a resistance mechanism causes antibiotics to fail to limit bacterial growth. The passive mechanism can only be transferred to other cells through clonal transfer, which includes changes to the target site or a reduction in antimicrobial absorption without changing the antibiotic composition. Intrinsic resistance is another name for this type of resistance. The active method, on the other hand, includes lowering the intracellular antibiotic concentration by modifying or degrading its structure using

enzymes or by the action of efflux pumps (Martinez and Baquero, 2014).

2.9.1 Intrinsic resistance

Intrinsic resistance is a bacterium's innate ability to withstand the effects of antibiotics as a result of alterations in its physiological condition. Intrinsic resistance has a minimal probability of dissemination between bacterial genera because resistance genes are located in the chromosome with little transference to other genus. If it is flanked by insertion sequences that facilitate its mobilization, any gene responsible for intrinsic resistance could be spread and passed to other bacteria (Mathur and Singh, 2005).
Intrinsic resistance mechanisms presented by LAB include
• The alteration of the cell wall, which is typically seen in resistance to glycopeptides (vancomycin and teicoplanin) and non-ribosomal antibiotics (bacitracin). Lactobacillus plantarum and Enterococcus faecium, in particular, have innate resistance to vancomycin, owing to the substitution of D-alanine residues in the muramyl pentapeptide cell wall by D-lactate (high-level resistance) or D-serine (low-level resistance) in the peptidoglycan's chemical structure, avoiding antibiotic interaction (Munita and Arias,2016).
• Enzymatic inactivation of antibiotics such as aminoglycosides (neomycin, streptomycin) or quinolones (norfloxacin, nalidixic acid) prevents these antibiotics from binding to their specific targets, as seen in Lactobacillus and Enterococcus for the 16S rRNA of the 30S ribosomal bacterial subunit and DNA gyrase, respectively, which explains intrinsic resistance to both (Jaimee and Halami, 2016).

2.9.2 Extrinsic resistance

• Extrinsic or acquired resistance refers to the ability of bacteria to incorporate antibiotic resistance into their cellular structure. Horizontal gene transfer (HGT) happens when bacteria acquire additional genes that can broaden their resistance spectrum or transfer resistance to other bacteria. Transduction (through bacteriophages) and transformation (when DNA is released from one bacterium and absorbed by another) are the two main methods of HGT, with conjugation being the most common among lactic acid bacteria. 2014 (Huddleston).

2.10 Conjugation in LAB

Conjugation is a method of transferring genetic material from plasmids or transposons to sexual pilus via a clumping factor and the lax gene. Plasmids are extrachromosomal DNA molecules that give antibiotic resistance to bacteria and propagate resistant genes for antibiotics such as -lactams, aminoglycosides, tetracyclines, chloramphenicol, sulphonamides, trimethoprim, macrolides, and quinolones. Plasmids can be used to give resistance by conjugation, and a single bacteria can have numerous plasmids. Although transposons and integrons do not self-replicate and must be delivered by a suitable plasmid or phage, the genetic diversity of resistance is related to the number of plasmids present in the environment. Tn916, Tn918, Tn920, Tn925, Tn2702(E. faecalis), Tn5233(E. faecium), Tn5276, and Tn5301 are some conjugative transposons employed as vehicles for antibiotic resistance genes in LAB (Sharma et al., 2014).

2.11 Evaluation of AMR Transmission from LAB to Potential Foodborne Pathogens

Using the filter mating approach, the conjugal transfer rate AMR from LAB to pathogens was determined. Using a Millipore pump set at 50 kPa, one milliliter of donor culture and one milliliter of recipient culture were filtered through a sterile Millipore membrane filter (2.5-cm diameter, 0.45-mm pore size) (MF-Millipore membrane filter). 10mL of sterile peptone physiological saline solution (PPS) was poured through the filter after the donor and recipient cells were filtered. Filters were put on GM17 agar aseptically, cell side up. Plates were incubated at 300°C overnight. Following incubation, filters were placed in 2mL of PPS, with an additional 1mL of PPS used to wash the mating plate, and the washings were placed in a sterile tube with the original mating filter (3mL final volume PPS). Filters were vortex-mixed to dislodge all cells, and repeated 10-fold dilutions were plated onto GM17 media that was selective for donors, recipients, and transconjugants. Plates were incubated at 30°C for 48 hours (Toomey et al.,2009).
The antimicrobial resistance (erythromycin or tetracycline) transferability between 47 mating pairs was tested using the filter mating method. The erythromycin resistance [erm(B)] gene transfer from Lactococcus lactis SH4174 and Streptococcus thermophilus E2 to Staphylococcus aureus (3), Listeria monocytogenes (H7), L. monocytogenes (L1), L.welshimeri, E. coli K12 (597), E. coli K12 (626), and Lactococcus lactis BU-2-60 was investigated (control). Both LAB donors, Listeria monocytogenes and L. welshimeri, and the control LAB Lactococcus lactis BU-2-60 strain, showed transfer. The number of transconjugants transferred from LAB to Listeria spp. ranged from 2.1x10-8 to 5.1x10-4 per recipient. There was no erm(B) transmission between LAB and S. aureus or E. coli.
Thirty-three mating pairs investigated tetracycline resistance transfer, with 11 mating pairs focusing on tet(M) transfer from Lactococcus lactis IBB477 and 22 mating pairs investigating tet(S) transfer from Lactococcus lactis IBB160 and Streptococcus thermophilus T3 to Staphylococcus aureus (3), S. aureus (7-9), Listeria monocytogenes (There was no tetracycline resistance transfer between LAB and S. aureus, Listeria, E. coli, or Salmonella spp., either tet(M) or tet(S). Tet(M) transmission from Lactococcus lactis IBB477 to E. faecalis was found, whereas tet(S) transfer was not. Both tet(M) and tet(S) markers were transferred to the control strain L. lactis BU-2-60.

They came to the conclusion that the most serious issue with resistance transfer between LAB or non-pathogenic commensal species is the risk of transferring the same resistance determinants into pathogenic strains, extending the resistance profile of these bacteria and complicating infection treatment (Toomey et al.,2009).

2.12 European standards

The FEEDAP Panel establishes microbiological cut-off values to identify resistant from susceptible strains. The distribution of MICs of the chosen antimicrobials in bacterial populations belonging to a particular taxonomic unit is used to determine microbiological cut-off values (species or genus). The population that clearly differs from the normal vulnerable populations is classified as resistant. The information used to determine microbiological cut-off values came from published studies, the European Committee on Antimicrobial Susceptibility Testing, and national and European monitoring programs. Strains of bacteria used as feed additives can be classified as susceptible to antimicrobials or resistant to antimicrobials (EUCAST, http://www.eucast.org/).

❑ **Microbiological cut-off values for LAB**

• Susceptible (S): When a bacterial strain is inhibited at a concentration of a specific antibiotic equal to or less than the stated cut-off value (S x mg/L), it is said to be susceptible.

• Resistant (R): A bacterial strain is resistant when it is not inhibited by a specific antimicrobial concentration greater than the prescribed cut-off value (R > x mg/L).

Species/group	Ampicillin	Vancomycin	Gentamicin	Kanamycin	Streptomycin	Erythromycin	Clindamycin	Tetracycline	Chloramphenicol
Lactobacillus obligate homofermentative	1	2	16	16	16	1	1	4	4
Lactobacillus acidophilus group	1	2	16	64	16	1	1	4	4
Lactobacillus obligate heterofermentative	2	n.r	16	32	64	1	1	8	4
Lactobacillus reuteri	2	n.r	8	64	64	1	1	16	4
Lactobacillus fermentum	1	n.r	16	32	64	1	1	8	4
Lactobacillus facultative heterofermentative	4	n.r	16	64	64	1	1	8	4
Lactobacillus plantarum/pentosus	2	n.r	16	64	n.r	1	1	32	8
Lactobacillus rhamnosus	4	n.r	16	64	32	1	1	8	4
Lactobacillus casei/paracasei	4	n.r	32	64	64	1	1	4	4
Bifidobacterium	2	2	64	n.r	128	1	1	8	4
Pedicoccus	4	n.r	16	64	64	1	1	8	4
Leuconostoc	2	n.r	16	16	64	1	1	8	4
Lactococcus lactis	2	4	32	64	32	1	1	4	8
Streptococcus thermophilus	2	4	32	64	64	2	2	4	4
Propionibacterium	2	4	64	64	64	0.5	0.25	2	2

(n.r- not required)

The cut-off values identified should be seen as a pragmatic response intended to introduce consistency in the separation of strains with acquired resistance from susceptible strains.

2.13 ABR in LAB utilized in fermented foods determination:

GRAS and QPS classifications have been conferred to LAB since it has been demonstrated that these bacteria can exchange genes to improve their survival in antibiotic-containing environments and can transfer genes across bacteria of different genera in the intestine, both commensal and pathogenic species.

2.13.1 Isolation of lactic acid bacteria from cheese samples

Isolated LAB from cheese samples were homogenized in 90 mL sterilized buffered peptone water, serial dilutions (10-1 to 106) were conducted, and parts (0.1 mL) from each dilution were plated onto MRS agar, agar, and Kanamycin Esculin Azide Agar (KAA) plates. Under anaerobic circumstances, M17 and MRS plates were incubated at 30°C for 48 hours, and KAA plates were incubated at 37°C for 24 to 48 hours (5 percent CO2). Following incubation, 74 isolates/colonies were chosen at random from MRS agar, M17 agar, and KAA plates and purified three times by sub-culturing onto the appropriate MRS medium (Kanak and Yilmaz, 2020).

2.13.2 Characterization of LAB's microbiological and biochemical properties

Cell shape, Gram reaction, and catalase production were all examined on 74 pure bacterial isolates. Only Gram-positive and catalase-negative isolates were used in future experiments, and 36 isolates were evaluated for growth at varied NaCl concentrations (4 and 6.5 percent), temperatures (30 and 45 degrees Celsius), and pH values (9.2 and 9.6) [Halkman,2005].

2.13.3 Kirby-Bauer Disk diffusion method for determining antibacterial activity of LAB

On MRS agar, 36 LAB isolates were injected in total. Using McFarland Biosan 1B, the cell concentration of the isolate was adjusted to a density of 0.5-0.6 McFarland (107cfu/mL). For 48 hours, 20 mL sterile MRS broth supplemented with 1% isolate was incubated at 30 °C. The isolates were centrifuged for 45 minutes at 6000 g at 4 °C after incubation. Using sterile membrane filters with a pore width of 0.22 m, supernatants were sterilized. Listeria monocytogenes ATCC 7644, Staph. aureus ATCC 25923, E. coli O157: H7, Cit. sakazakii ATCC 29544, B. cereus, and Salmonella typhimurium ATCC 140828 were also injected into TSA and incubated at 37 °C for 24 hours. The test microorganisms were disseminated over TSA with a cell concentration of 107cfu/mL. After that, 15 liters of supernatants were poured onto the discs (dia-6mm). The petri dishes were incubated at the temperature recommended for each indicator pathogen for 24 hours. Zones of inhibition surrounding the discs in each plate were measured in millimeters after 24 hours. The antibacterial activity of 36 LAB isolated from cheese was studied in this study. During the research, 18 isolates were shown to have antibacterial activity (Yamato et al., 2003).

Antibacterial activity was best against E. coli O157:H7 (19 mm) and S. Typhimurium ATCC 140828 (13 mm), L. monocytogenes ATCC 7644 (14 mm), and C. sakazakii ATCC 29544 isolates (17 mm). Antimicrobial activity was found in about half of the LAB against six infections. Antimicrobial activity of the Lc. garvieae isolate (A77) against L. monocytogenes ATCC 7644, E. coli O157:H7, C. sakazakii ATCC 2954, S. Typhimurium ATCC 140828, and Staph. aureus ATCC 25923 was excellent. Antimicrobial activity of Lc. lactis isolate (B3A) against C. sakazakii ATCC 29544 was strong (17 mm). Antimicrobial activity against E. coli O157:H7 was highest in the Lb. plantarum isolate (AS5). Antimicrobial activity of the Lb. plantarum isolate (G5S) was likewise extremely good against L. monocytogenes ATCC 7644, C. sakazakii ATCC 2954, and Staph. aureus ATCC 25923. Antimicrobial activity against B. cereus was not found in any of the isolates. The pathogenic strains were successfully suppressed by the isolated LAB, demonstrating that include LAB in commercial food products can provide effective protection against infections caused by these bacteria (Yamato et al., 2003).

2.13.2 Determination of ABRinLAB

Antibiotic susceptibility of isolated isolates was tested using the agar disc-diffusion method. The bacteria were cultured in MRS broth for 24 hours at 30 degrees Celsius, and then 200 liters of each culture were put on MRS agar plates. On the plates, antibiotic standard discs were inserted. The test included paper discs containing vancomycin (VA, 30 mg), chloramphenicol (C, 30 mg), rifampicin (RA, 5 mg), tetracycline (TE, 30 mg), erythromycin (E, 15 mg), nitrofurantoin (F, 300 mg), gentamicin (CN, 10 mg), and ciprofloxacin (CIP, 5 mg). The NCCLS document M2-A9 criteria were used to evaluate bacterial strains. 13 of the 18 LAB isolates examined had rifampicin resistance, 6 had tetracycline and vancomycin resistance, 5 had erythromycin and nitrofurantoin resistance, and 1 had gentamycin, chloramphenicol, and ciprofloxacin resistance. Rifampicin resistance was discovered in 72.2 percent of the strains, 53.3 percent of the ones resistant to tetracycline and vancomycin, and 27.7% of the strains resistant to erythromycin and nitrofurantoin. These findings back with the theory that foodborne bacteria could be a source of antibiotic resistance genes (Kanak and Yilmaz, 2020).

2.14 Evaluation of ABR in LAB from Dahi

In Dahi, ABR was determined in a Probiotic LAB. They had collected 33 samples of domestic Dahi from various regions of North Bengaluru (Ramachandra et al., 2017).

2.14.1 Isolation and identification of LAB:

From the 33 domestic Dahi samples, 80 lactic nature isolates were chosen using the serial dilution technique, and after testing them in milk for curdling time, acidity, and Direct Microscopic Counts (DMC), only 21 lactic isolates performed reasonably well. Furthermore, according to Bergey's Manual of Systematic Bacteriology, these 21 lactic isolates were recognized to species level (2009). 16S r RNA sequencing was used to corroborate the phenotypically indicated 21 lactic isolates.

2.14.2 Determination of ABR in LAB by Disc diffusion method

Antibiotic susceptibility of isolates was tested using the disc diffusion method with antibiotic discs containing penicillin (10g), gentamycin (10g), streptomycin (10g), chloramphenicol (10g), kanamycin (30g), erythromycin (15g), and bacitracin (10g). Antibiotic discs were firmly adhered to the previously dried agar plates' surface. In one plate, antibiotic discs were inserted. Plates were incubated for 24 hours at 37 or 30 degrees Celsius, and the diameter of the zones created was measured with a calibrated scale. If the diameter of the zone formation, including the disc, is less than 10 mm, the isolate is deemed resistant, while isolates with a diameter greater than 10 mm are considered susceptible.
The antibacterial activity against selected pathogens was examined against the commonly used 7 antibiotics in selected Lactobacillus isolates from isolated Dahi samples that were identified to its probiotic property, i.e. acid, bile, and the antibacterial activity against selected pathogens. All of the isolates had various responses with different antibiotics, however all 13 lactobacilli isolates were susceptible to all seven medications since their inhibitory zones were greater than 10 mm in diameter.
They concluded that antibiotic resistance is strain, species, and antibiotic specific based on their findings.

Sl.No.	Name of Lactobacillus	Name of Antibiotic						
		Pen	Str	Gen	Kan	Chl	Ery	Bac
1	Lab. rhamnosusLB1	24.67	12.67	20.33	14.00	22.33	38.67	19
2	Lab.rhamnosusLB2	22.00	12.00	19	14.33	19.33	36.67	19.67
3	Lab. rhamnosusLB3	18.67	11.67	18	10.67	16.33	37.67	21.33
4	Lab. rhamnosusLB4	21.00	11.67	22	10.67	21.67	35.00	19.33
5	Lab. rhamnosusLB5	24.33	13.67	17.33	12.33	21.00	40.33	24.67
6	Lab.fermentumLB8	22.00	13.33	17.67	13.33	19.67	30.67	21.33
7	Lab.plantarumLB9	24.33	12.67	16	14.67	30.67	30.67	21
8	Lab.fermentumLB11	21.67	12.33	18	14.67	26.33	31.00	19
9	Lab.fermentumLB12	22.00	14.00	20.67	14.33	23.00	29.33	19.67
10	Lab.plantarumLB14	31.00	18.67	21.33	17.67	25.00	28.67	18
11	Lab.plantarumLB15	35.33	10.67	18	13.67	23.00	32.67	20.33
12	Lab.delbrueckiissp.bulgaricusLB20	22.33	12.33	22.33	13.00	20.67	29.67	22.33
13	Lab.delbrueckiissp.bulgaricusLB23	23.67	11.33	18.67	12.00	19.33	31.00	18.00

Pen-Penicillin (10µg), Str-Streptomycin (10µg), Gen-Gentamycin (10µg),Kan-Kanamycin (30µg), Chl-Chlroamphinicol (10 µg), Ery- Erythromycin (15µg), Bac-Bacitracin (10 µg)
All the values are mean of three trials

Table 1: Antimicrobial susceptibility testing (AMST) of the LAB.

2.15 Antimicrobial resistance of LAB in fermented food(Mathur and Singh, 2005)

Foods	Species	Resistance
Raw meat products		
Poultry	*Lb .reuteri*G4	cat
Raw ground meat	*Lb .reuteri*100-63	erm(T)
	*Lb plantarum cat*TC2R	Cm
Raw ground pork and beef	*Lb sakei,Lb.currvatus*	Tetracycline (69%)
	Lb plantarum,Lb brevis	chloramphenicol (3%)
	Ln.mesenteroides	methicillin (85%)
Fermented products		
Raw milk soft cheese	*Lc.lactis*strain K214	Str-tet(S)-cat
Greek cheese	*Lb.acidophilus*ACA-DC 243	Pencillin
Yoghurt starter cultures	*S.thermophilus*	Neomycn, polymyxin B
	*Lb.delbrueckii*ssp. *bulgaricus*	

Nigerian fermented foods and beverages	*Lb.pentosus, Lb.acidophilus,Lb.casei,Lb.brevis, Lb.platarum,Lb.jensenii*	Tetracycline (42.5%) Erythromycin(17.5%) Ampicillin(47.5%) Cloxacillin(80%) Pencillin(77.5%)
Fermented dry sausages	*Lactobacillus* spp.	Tetracycline Gentamicin (79%) Pencillin(64%) Kanamycin(79%)
Turkish yoghurts	*S.thermophilus*	Vancomycin(65%)
European probiotic products	*Lb.acidophilus,Lb.rhamnosus*	Tetracycline(26%)
	Lb.casei,Lb.johnsonni	Pencillin(23%)
	Lb.plantarum,Lb.reuteri	Erythromycin(16%)
	*Lb.delbreukii*spp. *bulgaricus*	Chloramphenicol(11%)
Others		
Maize silage	*Lb.plantarum*5057	Tet(m)

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

2.16 Requirements for the use of LAB

After evaluating general elements of safety, taxonomy, capacity to create pathogenicity toxins, antibiotic resistance, and the historical context of food safety, the FDA assigns microorganisms the GRAS designation. LAB has a long history of use in fermented foods, and they are generally considered safe. The spread of AR genes, on the other hand, puts the GRAS category in a different light, particularly for bacteria that have mobile genes of transfer, such as Lactobacillus, because there are still no guidelines in the United States that consider the type of resistance in microorganisms used in food processing.

Since 2003, the European Commission has regulated the safety of LAB used as starter or probiotic cultures in Europe through the European Food Safety Authority (EFSA), which has established guidelines for awarding qualified presumption of safety (QPS) quality to organisms. The word QPS refers to a concept that is based on reasonable and qualified evidence to enable some limits. It is similar to the GRAS idea, but with more stringent rules that ensure the bacteria's reliable safety, making the phrase "from farm to fork" apparent (Laulund et al., 2017)

2.16.1 Methodology for assessing LAB resistance to antibiotics used in food

The FEEDAP (Panel on Additives and Products or Substances Used in Animal Feed) Panel proposed a scheme to evaluate the resistance present in LAB that can be used as probiotics or starter cultures in food processing; it's critical to distinguish between intrinsic and acquired resistance as part of lactic acid bacteria's food safety (EFSA,2008,Laulund et al., 2017)

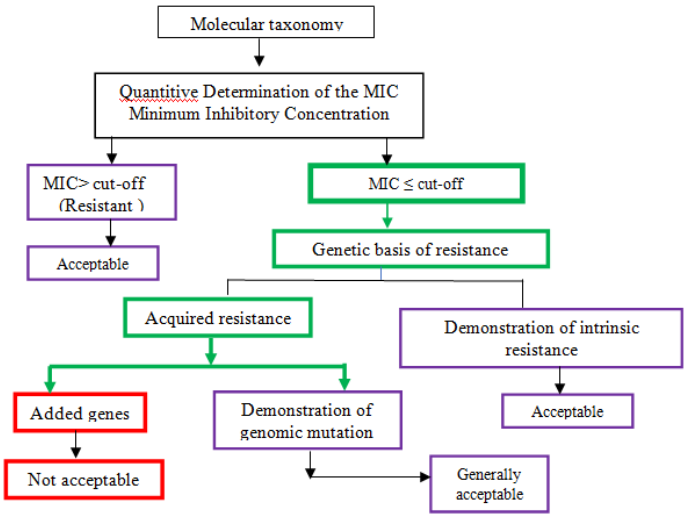


Fig 1: Proposed scheme for the ABR assessment of LAB used as probiotic and starter culture.

The correct identification of the bacteria (sequencing and comparison of the 16S rDNA gene in international databases) by molecular taxonomy is essential to evaluate the type of resistance, since the intrinsic resistance is specific for a specie or genus. Once the specie under study has been identified, the MIC in which the LAB is sensitive to the antibiotic analysed is determined. The bacterium can be considered safe when the MIC is lower than the cut-off level (MIC < cut-off). If the MIC value is above the cut-off value (MIC > cut-off), the bacterium is considered resistant to the antibiotic, and its resistance should be confirmed by molecular methods as PCR However, the resistance genes not always are expressed but can be transferred to other bacteria if the environmental conditions stimulate the expression of these genes. If the bacteria have intrinsic resistance, it is considered acceptable for use in food. Otherwise, it must be demonstrated whether the acquired resistance is in mobile genetic material or was acquired in the process of mutation in the bacterial chromosome (also acceptable for use in foods). Finally, the bacteria are not accepted by any regulatory body for its application in food if it is demonstrated that the resistance is exogenous and easily transferable (EFSA, 2008).

3. CONCLUSION

Antibiotics are the agents used to kill the bacteria. LAB are employed in preparation of fermented milk products .ABR in LAB has a Public Health Importance as ABR genes in LAB could horizontally transferred to plasmid or transposon genes of enteric pathogen, which is considered to be the biggest threat to global health. European committee has given standard reference cut-off values to LAB with respect to antimicrobial susceptibility testing (AMST). Standard and appropriate tests are required to identify the presence and transferability of ABR genes in LAB. Adaptation ofstandards in selection ABR in LAB is needed. Prevention and transfer of ABR gene of LAB to pathogens is required to maintain normal health of human beings.

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