Antibiotic Susceptibility of Lactic Acid Bacteria Isolated From Cow, Goat, Donkey, Buffalo, Sheep, Camel and Human Milk

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Abstract. Breast milk is a very important factor in regulating gastrointestinal function, improving the immune system and preventing acute illnesses (e.g. acute otitis media), especially during breastfeeding. Breast milk allows beneficial bacteria such as bifidobacteria and lactobacillus species to colonise the newborn intestin*e instead of potential enteropathogenic bacteria such as streptococci and *escherichia coli*. The aim of the study was to compare the antibiotic resistance of Lactic acid bacteria members isolated from different milk samples. Six milk samples were collected from each of seven different milk sources (donkey, goat, cow, buffalo, sheep, camel and human). The MRS and M-17 medium were used with the double layer sandwich method for isolation of LAB members selected from typic colonies, gram-positive, catalase-negative used in the study. The isolated 42 LAB species were determined antibiotic susceptibility with 9 standart antibiotic discs such as tetracycline, penicillin, kanamycin, streptomycin, rifampin, gentamicin, chloramphenicol, teicoplanin, ciprofloxacin by agar disc diffusion assay test. The 18 LAB isolate were resistant to penicillin, 16 isolate to kanamycin, 14 isolate to gentamicin. LAB isolates such as H1, H4, H5 from Human milk and LAB isolate such as D1 from donkey milk was considered as MDR isolates because they were resistant to at least four of the tested drugs. Among the milks, sheep, camel, buffalo and goat milks were found to be more sensitive to antibiotics on average in the group.

Key words: Lactic acid bacteria, Antibiotic resistance, Cow milk, Goat milk, Donkey milk, Buffalo milk, Sheep milk, Camel milk, Human milk

1. Introduction

Milk is the sole food of newborn mammals, and complex colloidal dispersion composed of fats, proteins, lactose, minerals, microorganisms and other minor compounds. Breast milk is a very important factor in regulating gastrointestinal function, improving the immune system and preventing acute illnesses (e.g. acute otitis media), especially during breastfeeding [1]. Ninety per cent of the milk available in the market is cow's milk and 10 per cent of the milk consists of other milk types. For this reason, especially in infant nutrition, the search for the closest milk to human milk continues. In addition to growth and development, milk has many important properties in the life cycle due to its protein and peptide structured elements such as immunoglobulins, enzymes, enzyme inhibitors, growth hormones, other hormones, growth factors, antibacterial agents, fatty acids, vitamins and minerals, which are physiologically important. Species used for food fermentation generally belong to the genus *Lactococcus, Streptococcus, Pediococcus, Leuconostoc, Lactobacillus* and newly recognized Carnobacterium. These organisms are isolated from cereals, green plants, milk and meat products, fermented vegetables and mucosal surfaces of animals [2]. Lactic acid bacteria used in fermented food production and as probiotics are generally considered safe microorganisms and are expected to suppress the number of pathogens in the intestinal flora. Breast milk allows beneficial bacteria such as bifidobacteria and lactobacillus species to colonise the newborn intestine instead of potential

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enteropathogenic bacteria such as streptococci and *escherichia coli (*Beattie, Pannaraj).

The fact that Lactic acid bacteria have been popular as probiotic in food production in recent years has been seen as one of the most natural ways to naturally achieve and spread antimicrobial resistance. Since the main threat is to transfer lactic acid bacteria to resistance genes to pathogenic bacteria, post-modern studies are investigating the reservoir roles of lactic acid bacteria from commensal bacteria for the antibiotic resistance gene [3]. However, LAB species ability to transfer antibiotic resistance genes to pathogens makes them an important threat beyond innocent. Therefore, care should be taken that lactic acid bacteria to be used in the food industry do not carry resistance genes, and disciplines that limit the use of unconscious and unnecessary antibiotics are needed. Another problem is the development of new antibiotics or combined methods to combat pathogens [4,5,6]. Multi-drug resistance (MDR) is a major problem in chemotherapy. In solving this problem, it is mandatory to determine the multidrug resistance pattern of an isolate [7]. Increasing the number of multi-drug resistant (MDR) pathogens is currently a serious problem all over the world. The defence mechanism of microorganisms against antibiotics used in the treatment of many diseases poses a major problem for humanity [8]. The aim of the study was to compare the antibiotic resistance of Lactic acid bacteria members isolated from different milk samples. Milk sources such as donkey, goat, cow, buffalo, sheep, camel and human. It was also aimed to determine the multidrug resistance (MDR) of 42 LAB species isolated from seven milk sources.

2 Material and Method

In our study, 6 pieces of milk were collected from 6 different milk sources such as donkey, goat, cow, buffalo, sheep, camel and human, and brought to the laboratory with the cold-chain procedure. After the raw milk samples are homogenized in 10 ml sample/90 ml Ringer's solution of each sample, 10^{-3} -10^{-6} dilutions are prepared. MRS and M-17 medium were used with the double layer sandwich method for isolation of LAB members. Creamy smooth developing in MRS and M-17 agar petri dishes were randomly selected from typical colonies in colonies morphology, other procedures were implemented. Then Typical colonies morphology such as creamy, smooth on MRS and M-17 agar petries then showing a range of 3.5-5.0 pH for MRS broth, gram-positive, catalase-negative were selected as Lactic acid bacteria species. 42 LAB species from milk samples tested their resistance to antibiotics with 9 different standard antibiotic discs. LAB members are revived in stock cultures M17 and MRS liquid feeds, and their reproductive densities are set in 0.5 Mac mice according to Kirby Bauer Disc Diffusion method [9]. Later, 0.1 ml of MRS agar was infused from fresh cultures in MRS Agar and M17 Agar feeds and spread homogeneously. Then commercial antibiotic paper discs to the surfaces of petri boxes that are cultivated from cultures, chloramphenicol (30 µg CT 013 B), gentamicin (10 µg-CT 024 B), penicillin G (10 units), streptomycin (5 µg), and tetracycline (30 µg), kanamycin 30 µg, teicoplanin (30 µg CT 647 B rifampin (30 µg- CT 104 B) was placed with the help of dispenser at appropriate intervals (figure 3.1). Results as inhibition zone area of isolates against antibiotic dics were valued by Clinical and Laboratory Standards Institute CLSI [10] were.

Each LAB member isolates were treated with antibiotic discs and inhibition zone diameters (in mm) were measured at the end of the incubation period and each isolate was interpreted as resistant, semi-resistant and susceptible. The results were evaluated according to Clinical and Laboratory Standards Institute CLSI 2011 [11]. Multiple drug resistance (MDR) is the ability of some microorganisms to resist multiple antimicrobial effects. It was also aimed to determine the multidrug resistance (MDR) of 42 LAB species isolated from milk. Calculation of Multiple Antibiotic Resistance (MAR) Index Multiple antibiotic resistance (MAR) index was calculated as where a represents the number of antibiotics to which the isolates were resistant and ‘b’ represents the total number of antibiotics to which the isolate was exposed [11].

3 Result

Lactic acid bacteria isolated all of milk samples such as human, goat, cow, buffalo, camel, sheep, and donkey with MRS and M-17 selective medium. The LAB isolates were evaluated antibiotic resistance by 9 antibiotic dics.

Table 1, 9 antibiotic resistance and sensitivity test results of LAB species isolated from each milk sample are given.

MDR is the multiple resistance of microorganisms against drugs such as antifungal, antiviral and anti-parasite. Certain chemicals have similar inhibitor effects on some microorganisms, such as normally killing them or limiting their growth. Identification of Multidrug Resistance (MDR) Strains, the number of antibiotics each bacterium was resistant to in the disc diffusion test was noted for identification of multidrug-resistant strains. Multidrug resistance (MDR) was taken as resistant to four or more antibiotics tested [12].

The isolates were mostly susceptible tetracycline, penicillin, kanamycin, streptomycin, rifampin, gentamicin, chloramphenicol, teicoplanin, ciprofloxacin

antibiotics. Same isolates were found resistant on penicilin G.

Table 1. Antibiotic susceptilty of LAB species isolated from milk of human, camel, sheep, cow, buffalo, goat, donkey,

LAB isolate source	Antibiogram/standard antibiotic discs								
Human milk	TE-30	C-30	S-10	CN-10	TEC-30	RD-30	P-10	CIP-30	K-30
H-1	R	R	S	R	S	S	R	S	R
H-2	R	S	S	S	S	S	R	S	R
H-3	S	S	S	R	S	S	S	S	R
H-4	R	R	S	S	S	S	I	S	S
H-5	R	R	R	I	S	R	S	S	R
H-6	R	S	S	S	R	S	R	S	S
Camel milk									
CA-1	S	S	S	S	S	S	R	R	S
CA-2	S	S	S	S	S	S	I	S	R
CA-3	S	R	ND	S	S	R	S	S	R
CA-4	S	S	S	S	S	S	R	S	R
CA-5	S	S	S	S	ND	S	R	R	S
CA-6	S	S	S	S	S	S	R	I	ND
Sheep milk									
S-1	S	S	ND	S	S	S	R	S	S
S-2	S	S	R	S	S	S	R	S	R
S-3	S	S	R	S	S	S	I	S	S
S-4	S	ND	S	S	S	S	R	S	R
S-5	S	S	R	S	S	S	I	S	S
S-6	S	R	S	S	S	S	ND	S	S
Cow									
C-1	S	S	R	S	S	S	R	S	S
C-2	S	S	S	S	S	S	R	S	S
C-3	S	S	S	S	S	S	R	R	ND
C-4	S	S	S	S	S	S	R	S	S
C-5	S	S	I	S	S	S	S	R	S
C-6	S	S	S	S	S	S	R	S	S
Buffalo milk									
B-1	S	R	S	S	S	S	S	S	S
B-2	S	R	S	I	S	S	S	S	S
B-3	S	S	ND	S	S	S	R	S	S
B-4	S	S	ND	S	S	S	S	S	S
B-5	S	S	R	S	R	S	S	I	S
B-6	S	S	R	I	R	S	R	S	S
Goat milk									
G-1	S	R	S	S	S	S	S	S	S
G-2	S	S	ND	S	S	S	S	R	R
G-3	R	R	S	S	S	R	S	S	S
G-4	S	R	S	S	S	I	S	S	I
G-5	S	S	ND	S	S	S	S	R	R
G-6	R	R	S	S	S	R	S	S	S
Donkey milk									
D-1	S	S	R	R	S	S	I	S	R
D-2	S	S	R	S	S	S	S	S	S
D-3	S	S	S	S	S	S	I	S	R
D-4	ND	S	S	S	S	S	R	S	S
D-5	R	S	S	S	R	S	S	S	R
D-6	S	S	R	S	S	S	I	S	R

Antibiotic resistance degree was (R,I,S). R: resistance, I: intermediate, S: susceptible. R,I,S zone diameter value ranges were determined by taking NCBI for each antibiotic agent . Tetracycline TE-30: (14 or less; 15-18; 19 or more), Chloramphenicol C-30:(12 or less;13-17;18 or more), Rifampin:RD-30: (16;17-19;20 more), Gentamicin: CN-10:(12; 13-14; 15 more), Penicillin G :P-10: (11-12 ; 21; 22 more), Kanamycin:K-30: (12 less; 13-17; 18 more), Teicoplanin :TEC-30: (15 less; -; 28 more), Streptomycin:S10: (11; 12 -14; 15 more). CLSI, 2011

Of the total 42 LAB isolates, 18 were found resistant to penicillin, 11' chloramphenicol antibiotic, and 10 were resistant to streptomycin. Gentamicin, teicoplanin, and rifampin were found to be resistant to e 4 isolates, while 6 isolates were found resistant to cirproflaxin.

Table 2. Resistance pattern of LAB species isolated from raw milk (n=42)

Milk source	LAB isolate	Multiple antibiotic resistance Number number	MDR index	MAR Antibiotics
Human	H-1	5(9)	0.6	TE. C,CN,P,K
	H-2	3(9)	0.3	TE,P,K
	H-3	2(9)	0.2	CN,K
	H-4	3(9)	0.3	TE, C,P,K
	H-5*	6(9)	0.7	TE, C, S, CN, RD, K
	H-6	3(9)	0.3	TE,TEC,P,
Camel	CA-1	2(9)	0.2	P,CIP
	CA-2	1(9)	0.1	K
	CA-3	3(9)	0.3	C,RD.K
	CA-4	2(9)	0.2	P,K
	CA-5	2(9)	0.2	P,CIP
	CA-6	2(9)	0.2	P
Sheep	S-1	1(9)	0.1	P
	S-2	3(9)	0.3	S,P,K
	S-3	2(9)	0.2	S,P,K
	S-4	2(9)	0.2	P,K
	S-5	2(9)	0.2	S,K
	S-6	1(9)	0.1	C
Cow	C-1	2(9)	0.2	S,P
	C-2	1(9)	0.1	P
	C-3	2(9)	0.2	P,CIP
	C-4	1(9)	0.1	P
	C-5	3(9)	0.3	S,P
	C-6	1(9)	0.1	P
Buffalo	B-1	1(9)	0.1	C
	B-2	1(9)	0.1	C
	B-3	1(9)	0.1	P
	B-4	0(9)	0.0	P
	B-5	3(9)	0.3	S,TEC,CIP
	B-6	3(9)	0.3	S,CN,P
Goat	G-1	1(9)	0.1	C
	G-2	2(9)	0.2	CIP,K
	G-3	3(9)	0.3	TE,C,RD
	G-4	3(9)	0.3	C,RD,K
	G-5	2(9)	0.2	RD,K
	G-6	3(9)	0.3	TE, C, TEC
Donkey	D-1	4(9)	0.4	S,CN,P,K
	D-2	1(9)	0.3	S
	D-3	2(9)	0.1	P,K
	D-4	1(9)	0.2	P
	D-5	3(9)	0.1	TE,TEC,K
	D-6	2(9)	0.3	S,P,K

Multidrug resistance (MDR) = a/b. a: number of antibiotics to which the isolate is resistant, b: total number of antibiotics administered Antibiotic Tetracycline :TE, Chloramphenicol : C-30 Rifampin:RD, Gentamicin: CN, Penicillin G :P, Kanamycin: K, Teicoplanin :TEC, Streptomycin:s. CLSI, 2011.

LAB isolate from human milk maximum MDR value 0.7 (resistant to TE, C, S, CN, RD, K antibiotics) 0.6 (for h1> resistant to TE, C, CN,P,K antibiotics) calculated. Human milk isolate such as H1, H4, H5, donkey milk isolate such as D1 were found as MDR Because of 4 or 6 antibiotic resistance. Isolates were found to be resistant to at least two antibiotics, MDR value 0.2. The MDR value of 4' of 6 LAB species isolated from camel milk was found as >0.1. They were found particularly resistant to penicillin antibiotic.
The MDR value of 4 members of LAB species isolated from sheep milk was found as >0.1. 4 isolates were found resistant to kanamycin. MDR value of LAB members isolated from cow and buffalo milk was found at least three isolates with 0.1 or single antibiotic resistance. Two LAB isolates from the buffalo milk were found resistant to MDR value 0.3, three antibiotics. Three LAB isolates from cows milk were found resistant to S, P and CIP antibiotics with an MDR value of 0.2. MDR value of three LAB types isolated from goat milk 0.3 (especially resistant to C, RD, TE antibiotics) and two isolate was found to be 0.2 (of both, particularly resistant to K antibiotic). LAB isolates from donkey milk were found as the highest 0.4 and 0.3 in MDR. These isolates were also found particularly resistant to P antibiotic.

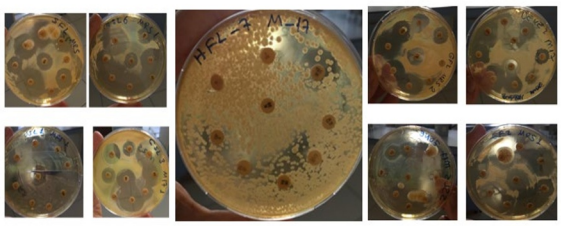

Fig. 1. LAB isolates from Camel, Human, goat, buffalo, sheep, cow, and donkey milk, were sensitive or resistant to all antibiotics tested, but this result was only for one isolate.

LAB isolates such as H1, H4, H5 from Human milk and LAB isolate such as D1 from donkey milk was considered as MDR isolates because they were resistant to at least four of the tested drugs. The isolates were mostly susceptible tetracycline, penicillin, kanamycin, streptomycin, rifampin, gentamicin, chloramphenicol, teicoplanin, ciprofloxacin antibiotics. Some isolates were found resistant on penicilin G.

4 Discussion

LAB strains often have the characteristics of GRAS status (Generally Recognised as Safe) certificates provided by the US Food and Drug Administration (FDA) and Qualified Safety Presence (QPS status) within Europe. Their therapeutic and prophylactic properties are important for the popularity of probiotics. LAB strains are under continuous investigation for their beneficial health properties for human and animal consumption and their safety for consumption as feed.

They are considered safe for human and animal consumption and the environment by the European Food Safety Authority (EFSA), the Panel on Biological Hazards (BIOHAZ) [13]. Today, some LAB strains [14]. Have gained resistance to antimicrobials used in human treatment and veterinary medicine. Resistance genes owned by LAB strains are considered as a reservoir that can be transferred to pathogenic bacteria. This phenomenon, which caused the spread of phenomena Antibiotic resistance among pathogens, has become a major problem as it makes treatments difficult [15]. Antimicrobial resistance (AMR) is considered an important safety issue when LAB is evaluated and approved as feed additives EFSA 2018 [16].
Although LAB species are widely considered safe and beneficial, cases of serious infections caused by LAB have been reported very rarely. These infectious diseases include bacteraemia [17-20] endocarditis [21], pleuropneumonia [17, 22], meningitis (1> urinary tract infections) 1> 1.

Antibiotic resistance of *Lactobacillus plantarum, Lactobacillus paracasei, Lactobacillus reuteri* and *Lactobacillus acidophilus* in foods has been reported in several studies [23]. The first study showing that starter cultures carry pathogenicity-related genes showed that starter cultures isolated from yogurt in China carry the aph (3') -IIIa and ant (6) genes. Therefore, studies on these issues are very important for public health. Intrinsic streptomycin resistance has previously been reported in lactobacilli, streptococci, lactococci and *Leuconostoc* sp. [24]. Thus, streptomycin resistance in these genera may be an intrinsic trait and can be considered as very low risk as a transfer without a specific safety concern [24]. According to this result, the intensity of streptomycin resistance in our isolates in general in our species may be an intrinsic feature in the same way as the intensity in LAB members, and in this case, the degree of risk would be low. Stefańska and others [25] emphasized that the first and key step in evaluating LAB strains as feed additives is to consider antibiotic resistances, so other in the selection of the best LAB strains to be used as feed additives they reported that it was a priority and important from complex studies. consequently, revising the existing microbiological cut-off values within the genus Lactobacillus and *Pediococcus* in the selection of the best LAB strains to be used for feed additives.

5 Conclusion

There is a significant risk of antibiotic resistance moreover MDR in dairy products in accordance with natural food trends. LAB members are also used to prolong shelf life with alternative protective properties as well as being the starting cultures of foods. It is important to determine antibiotic resistance in LAB members used in the food industry for this purpose or in

search of new LAB and LAB members with medical use. Antibiotic intake is known to provide selective pressure, resulting in a higher prevalence. Therefore, the causes of antibiotic resistance in humans and animals should be investigated, the effect of which should be given antibiotics to humans and antibiotics should be added to animal feed.

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