International Journal of Advanced Smart Convergence Vol.11 No.4 227-239 (2022)
http://dx.doi.org/10.7236/IJASC.2022.11.4.227

IJASC 22-4-25

H_2O_2 Generating Ability and Multi-Drug Resistance of Lactic Acid Bacteria Required for Long-Term Inpatient Treatment with Antibiotic Resistance

Yuk, Young Sam

Lecturer, Graduate School of Business (Health), Dankook University, Yong-in 16890, Republic of Korea
E-mail: y60320@gmail.com

Abstract

Purpose: In our study, in order to find lactic acid bacteria (LAB) with multi-drug resistance to antibiotics, we isolated 140 strains from 15 types of kimchi commercially available in Korea and 20 types of Kimchi made at home from January to December in 2016, and investigated their H_2O_2 generating ability and multi-drug resistance to antibiotics.

Methods: In order to observe the H_2O_2 generation ability of LAB, we performed the experiment with methods such as Rabe, Hillier, and Kang. To test the antibacterial susceptibility of LAB, we used the disc agar diffusion method using MRS agar (Difco, USA) according to the CLSI and WHO test methods.

There are 18 types of antibiotic discs used.

Results: Out of the total numbers of 140 strains, 6 strains of Ent. *Faecium*, 25 strains of *L. plantarum*, 1 strain of L. *rhamnosus*, 3 strains of L. *sakei*, 1 strain of *L. acidophilus*, 1 strains *St. thermophilus*, and 7 of unidentified strains generated H_2O_2 . The antibiotic susceptibility of *Ent. Faecium* indicated SXT, OX, NA, and E; and the antibiotic susceptibility of *L. plantarum* indicated NA; and the antibiotic susceptibility of *St. thermophilus* indicated NA, CC, RA, CTT, CM, and P ; and the antibiotic susceptibility of *L. rhamnosus* indicated SXT, VA, NA and CTT; and the antibiotic susceptibility of 6 strains of *L. sakei* indicated SXT, OX, NOR, NA, CTT and CIP, all indicating antibiotic resistance. In the case of multi-drug resistance to antibiotics for 53 strains of *L. antarum*, 8-drug resistance was the most common with 25 strains, followed by 7-drug-resistant strains with 18 strains, 9-drug-resistant strains with 4 strains, 6-drug-resistant strains with 3 strains, 5-drug-resistant strains with 2 strains, and 17-drug-resistant strains with 1 strain. In the case of multi-drug resistance to antibiotics for *Ent. Faecium* 27 strains, 9-drug resistance was most commonly identified as 9 strains, 8-drug resistance was identified as 6 strains, 7- and 11 drug resistances were identified as 4 strains each, and 4- and 6-drug resistances were identified as 1 strain each.

Conclusion: *Ent. Faecium*, *L. plantarum*, *L. rhamnosus*, *L. sakei*, and *St. thermophilus,* shown to have anantibacterial activity in previous studies on LAB and shown to have and H_2O_2 generating ability, antibiotic resistance and multi-drug resistance in this study, are expected to be able to play an excellent role for long-term inpatients to use as an alternative to antibiotics and to cope with emerging antibiotic resistance.

Key words*: LAB, Multidrug resistance of Lactobacillus, H_2O_2, Antimicrobial susceptibility, Antibiotic resistance*

1. Introduction

Manuscript Received: November. 20, 2022 / Revised: November. 23, 2022 / Accepted: November. 26, 2022
Corresponding Author: y60320@gmail.com
Tel: +82-41-550-1451, Fax: +82-41-559-7934
Lecturer, Graduate School of Business (Health), Dankook University, Korea

The emergence and the spread of multi-drug resistance organism (MDRO) caused by the misuse and abuse of antibiotics by long-term inpatients has been causing serious problems in society and public health. In the case of microorganisms with antibiotic resistance, 68.5% of the microorganisms that cause infection in the human eyeball are resistant to antibiotics [1]. About 45% of Staphylococcus epidermidis, a microorganism in the human nasal cavity and skin, were found to be resistant to methicillin [2]. Gram-positive anaerobic cocci (GPAC), a normal microbial colony living on the skin and mucous membrane surface of the human body, is the bacteria that are invasive and cause mixed infections most frequently when the host's immunity weakens. The bacteria had resistance rates to clindamycin, tetracycline, and moxifloxacin of 14%, 31%, and 24%, respectively, and 11% of the total isolates were multidrug-resistant [3].

The exposure to antibiotics by infants was found to be a major cause of delayed development of microbial group [4, 5]. The newly emerged Candida auris is a new multidrug-resistant organism threatening the world [6]. To find ways to solve these antibiotic resistances, studies in various aspects are needed.

Lactic acid bacteria (LAB) inhibit Gardnerella vaginalis by producing H_2O_2, L-lactate, and D-lactate [7]. And it reduces the rate of vaginitis recurrence from 41% to 20.5% and increases the recurrence time by 28% [8]. And according to previous studies, LAB and LAB culture medium show the effect of inhibiting or killing pathogenic bacteria [9-11]. And LAB has been suggested as an alternative to solving multi-drug resistance to antibiotics.

Therefore, in this study, in order to find LAB with multi-drug resistance to antibiotics that could serve as a substitute for antibiotics or as an adjuvant when using antibiotics for long-term inpatients with high antibiotic exposure, we isolated 140 strains from 15 types of Kimchi commercially available in Korea and 20 types of Kimchi made at home, and investigated their H_2O_2 generating ability and multi-drug resistance to antibiotics, from January to December in 2016.

2. Materials and Methods

2.1 Materials

2.1.1 Test strains. Table 1 shows several LABs which were isolated from Kimchi for our test strains.

Table 1. Kimchi isolated LAB through 16S rRNA sequencing used in this study

Identification of probiotics	Nos. of isolates(%)
Lactobacillus plantarum (L. plantarum)	53 (37.9)
Enterococcus faecium (Ent. Faecium)	27 (19.3)
Lactobacillus rhamnosus (L. rhamnosus)	7 (5.0)
Lactobacillus sakei (L. sakei)	6 (4.3)
Leuconostoc mesenteroides (Leu. Mesenteroides)	2 (1.4)
Lactobacillus acidophilus (L. acidophilus)	1 (0.7)
Lactobacillus alimentarius(L. alimentarius)	1 (0.7)
Lactobacillus casei(L. casei)	1 (0.7)

Lactobacillus pentosus(L. pentosus)	1 (0.7)
Streptococcus thermophilus (St. thermophilus)	1 (0.7)
Unidentified	40 (28.6)
Total	140

In the previous study from January to December in 2016, we identified LAB isolated from 15 commercially available domestic Kimchi and 20 home-made Kimchi by 16S rRNA sequencing; and by using 53 strains of *L. plantarum*, 27 strains of *Ent. Faecium,* 7 strains of *L. rhamnosus*, 6 strains of *L. sakei,* 2 strains of *Leu. Mesenteroides,* 1 strain of *L. acidophilus,* 1 strain of *L. alimentarius,* 1 strain of *L. casei,* 1 strain of *L. pentosus,* 1 strain of *St. thermophilus* and 40 strain of the unidentified, frozen at -80℃ and stored dry, we performed a research in order to find out the H_2O_2 producing ability and multi-drug resistance to antibiotics.

2.1.2 Antibiotics. Table 2 shows the antibiotics used in multi-drug resistance studies.

Table 2. Antibiotics used in multidrug resistance studies

Antibiotics	Volume	Manufacturing company
Ampicillin (AM)	10μg	BBL (USA)
Oxacillin (OX)	1μg	BBL (USA)
Cephalothin (CF)	300 μg	BBL (USA)
Cefotetan (CTT)	30μg	BBL (USA)
Ciprofloxacin (CIP)	5μg	BBL (USA)
norfloxacin (NOR)	10 μg	BBL (USA)
Imipenem (IPM)	10μg	BBL (USA)
Erythromycin (E)	15μg	BBL (USA)
Gentamicin (GM)	10μg	BBL (USA)
Streptomycin (SM)	10 μg	BBL (USA)
Clindamycin (CC)	2μg	BBL (USA)
trimethoprim/sulfamethoxazole (SXT)	1.25/23.75μg	BBL (USA)
nalidixic acid (NA)	30 μg	BBL (USA)
Penicillin (P)	10U	BBL (USA)
Vancomycin (VA)	30μg	BBL (USA)
Chloramphenicol (C)	30μg	BBL (USA)
Rifampin (RA)	5μg	BBL (USA)
Tetracyclin (TE)	30μg	BBL (USA)

The antibiotics used in this study are ampicillin (AM, 10㎍), oxacillin (OX, 1㎍), cephalothin (CF, 300 ㎍), cefotetan (CTT, 30㎍), ciprofloxacin (CIP, 5㎍), norfloxacin (NOR, 10 ㎍), imipenem (IPM, 10㎍), erythromycin (E, 15㎍), gentamicin (GM, 10㎍), streptomycin (SM, 10 ㎍), clindamycin (CC, 2㎍), trimethoprim/sulfamethoxazole (SXT, 1.25/23.75㎍), nalidixic acid (NA, 30 ㎍), penicillin (P, 10U), vancomycin (VA, 30㎍), chloramphenicol (C, 30㎍), rifampin (RA, 5㎍), tetracycline (TE, 30㎍). And we used BBL (Becton Dickinson & Company, USA) products for all antibiotics.

2.2 Methods

2.2.1 Testing the H_2O_2 generating ability of LAB.

We conducted an experiment with the methods such as Rabe, Hillier, and Kang in order to observe the H_2O_2 generating ability of LAB [12, 13].

In order to confirm the H_2O_2 producing ability of LAB, we confirmed each test strain for homogeneity on MRS agar, perform streak plate technique on TMB-Plus culture medium, perform anaerobic *cultures* at 35°C for 2-3 days, and exposed the culture medium to air.

Because the horseradish peroxidase in the culture medium oxidizes TMB in the presence of H_2O_2, the H_2O_2 generating colonies turned blue. And we used the prepared culture medium in a week.

2.2.2 Testing Antimicrobial Susceptibility of LAB.

To test the antibacterial sensitivity of LAB, we used the disc agar diffusion method using MRS agar (Difco, USA) according to the CLSI and WHO test methods [14, 15].

As shown in Figure 2, there are 18 types of antibiotic discs used.

We inoculated a single colony formed by culturing pure-isolated LAB three times for 48 hours in MRS agar culture medium under conditions at 37°C under 10% CO_2, and inoculated a single colony into MRS broth (Oxoid, UK), cultured by shaking at 37°C in 10% CO_2 for 24 hours.

We diluted the concentration of the cultured bacterial solution to a standard turbidity of 0.5 on the MacFarland scale, applied it uniformly on MRS agar using a sterilized cotton swab, turned the plate in directions of left or right, applied once, and finished by rotating the plate around.

We left the spread plate at room temperature for 30 minutes to dry it, and then divided the 18 antibiotic disks into 3 plates using a disc dispenser (Becton Dickinson & Company, USA) and attached them to the surface of the culture medium.

We flipped the spread plate and incubated for 24-48 hours or anaerobic culture at 37°C under 10% CO_2 conditions, and then measured the size of the inhibitor for each antibiotic formed around the disk, and then determined multi-drug resistances [16, 17].

3. Results

3.1 H_2O_2 generating ability of LAB.

Table 3 shows the H_2O_2 generating ability of 140 LAB strains isolated from Kimchi.

Table 3. Distribution of H_2O_2 producing isolates in LAB isolated from Kimchi

Probiotics	Nos. of isolates	Nos. of H_2O_2-production isolates(%)
Ent. faecium	27	6(22.2)
L. acidophilus	1	-
L. alimentarius	1	-
L. casei	1	-
L. pentosus	1	-
L. plantarum	53	25(47.2)
L. rhamnosus	7	1(14.3)
L. sakei	6	3(50.0)
Leu. mesenteroides	2	-
St. thermophilus	1	1(100)
Unidentified	40	7(17.5)
Total	140	43(30.7%)

Out of the total of 140 strains, 43 strains (30.7%) produced H_2O_2. Based on the relationship between strains by type, 6 strains (22.2%) out of 27 strains of *Ent. Faecium*; 25 strains 47.2%) out of 53 strains of *L. plantarum;* 1 strain (14.3%) out of 7 strains of *L. rhamnosus;* 3 strains (50.0%) out of 6 strains of *L. sakei;* 1 strain of *L. acidophilus;* 1 strain of *St. thermophilus;* and 7 strains (17.5%) out of 40 unidentified strains produced H_2O_2. And the *L. alimentarius, L. casei, Leu. Mesenteroides, L. pentosus,* and *L. acidophilus* did not show the ability to generate H_2O_2.

3.2 Antimicrobial Susceptibility of LAB

3.2.1 Antimicrobial Susceptibility of *Ent. Faecium, L. plantarum,* and *St. thermophilus.*

The result of antimicrobial susceptibility of *Ent. Faecium, L. plantarum,* and *St. thermophilus* was as shown in Table 4.

Table 4. Distribution of resistant, intermediate and susceptible antibiotics of *Ent. Faecium, L. plantarum* and *St. thermophilus* isolated from Kimchi

Antibiotics	Nos. of isolates (%)								
	Ent. Faecium(n=27)			*L. plantarum*(n=53)			*St. thermophilus*(n=1)		
	R	I	S	R	I	S	R	I	S
Ampicillin	1(3.7)	4(14.8)	22(81.5)	1(1.9)	-	52(98.1)	-	-	1(100)
Streptomycin	25(92.6)	1(3.7)	1(3.7)	50(94.3)	2(3.8)	1(1.9)	-	-	1(100)
Sulfamethoxazole/ Trimethoprim	27(100)	-	-	9(17.0)	8(15.1)	36(67.9)	-	-	1(100)
Vancomycin	-	-	27(100)	50(94.3)	1(1.9)	2(3.8)	-	-	1(100)
Gentamicin	22(81.5)	-	5(18.5)	45(84.9)	-	8(15.1)	-	-	1(100)
Tetracycline	-	-	27(100)	2(3.8)	3(5.7)	48(90.6)	-	-	1(100)
Oxacillin	27(100)	-	-	32(60.4)	9(17.0)	12(22.6)	-	-	1(100)
Norfloxacin	3(11.1)	20(74.1)	4(14.8)	51(96.2)	-	2(3.8)	1(100)	-	-

Nalidixic acid	27(100)	-	-	53(100)	-	-	-	1(100)	-
Erythromycin	27(100)	-	-	4(7.5)	-	49(92.5)			1(100)
Cephalothin	10(37.0)	10(37.0)	7(25.9)	2(3.8)	14(26.4)	37(69.8)	-	-	1(100)
Clindamycin	20(74.1)	3(11.1)	4(14.8)	2(3.8)	19(35.8)	32(60.4)	1(100)	-	-
Rifampicin	17(63.0)	8(29.6)	2(7.4)	2(3.8)	3(5.7)	48(90.6)	1(100)	-	-
Cefotetan	15(92.6)	1(3.7)	1(3.7)	52(98.1)	-	1(1.9)	1(100)	-	-
Chloramphenicol	-	-	27(100)	1(1.9)	-	52(98.1)	1(100)	-	-
Penicillin	-	12(44.4)	15(55.6)	1(1.9)	28(52.8)	24(45.3)	1(100)	-	-
Imipenem	-	-	27(100)	1(1.9)	-	52(98.1)	-	-	1(100)
Ciprofloxacin	-	21(77.8)	6(22.2)	51(96.2)	-	2(3.8)	-	-	1(100)

R : resistant, I : intermediate, S : susceptible

In the case of antibiotic susceptibility of 27 strains of *Ent. Faecium*, they were all resistant to SXT, OX, NA, and E, and resistance to S and CTT were 92.6%, GM 81.5%, CC 74.1%, CTT 63.0%, CF 37.0%, and AM 3.7%, respectively , and there was no resistance to VA, TE, CM, P, IMP and CIP. In the case of intermediate resistance of antimicrobials, CIP was the highest at 77.8%, and NOR at 74.1%, P at 44.4%, CF at 37.0%, RA at 29.6%, AM at 14.8%, CC at 11.1%, CTT and S were at 3.7%, respectively. The remaining antimicrobials do not have any intermediate resistance.

Susceptibilities were 100% each for VA, TE, CM and IMP. And AM has the susceptibilities of 81.5%, P 55.6%, CF 25.9%, CIP 22.2%, GM 18.5%, NOR and CC have 14.8% , and RA 7.4%, respectively. And S and CTT have 3.7% each. There were no susceptibilities for SXT, NA and E.

In the case of antibiotic susceptibility of 53 strains of *L. plantarum*, all NAs showed resistances. The resistance was 98.1% to CTT, 96.2% to NOR and CIP, 84.3% to VA and S, 84.9% to GM,

60.4% for OX and 17.0% to SXT. The remaining antimicrobials showed less than 10% resistance.

The intermediate resistance was the highest at 52.8% to P, followed by 35.8% to CC, 26.4% to CF, and 15.1% to SXT, and the rest were less than 10%.

The susceptibility was the highest for AM, CM, and IMP at 98.1%; followed by E at 92.5%; TE, and RA, CF at 90.6%; CF at 69.8%; SXT at 67.9%; CC at 60.4%; P at 45.3%;, and OX at 22.6%. The remaining antimicrobial agents showed less than 10% of susceptibilities.

The antibiotic susceptibility of one strain of St. thermophilus was resistant to NA, CC, RA, CTT, CM and P, but was not resistant to all other antibiotics. We found that there was an intermediate resistance only to NA, and the rest of the antimicrobials did not show an intermediate resistance.

Susceptibility was found in AM, S, SXT, VA, GM, TE, OX, CF, IMP and CIP; but not in the remaining antimicrobial agents.

In the case of 40 unidentified bacterial strains, the susceptibility of antibiotics was shown to have a high resistance of 97.5% to TT and NA each. And the resistance was 95% to S; 90.0% to VA; 87.5% to NOR and CIP each; 72.5% to SXT, GM and OX each; 37.5% to CF; 35% to AM and CC each; 32.5% to E; and 30% to TE, RA, CM, P ad IMP each.

For intermediate resistances, P was the highest at 35.0%, followed by CF and RA at 20.0% each; OX and CC at 15.0% each; TE at 12.5%; AM and SXT at 7.5% each; and CIP at 2.5%, and the remaining antibacterial agents did not show any intermediate resistance. Regarding susceptibilities, IMP was the highest at 70%; E and CM at 67.5% each; AM and TE at 57.5% each; RA and CTT at 50.0% each; CF at 42.5%; P at 35.0%;

GM at 27.5%; SXT at 20.0%; CIP at 15.0%; OX and NOR at 12.5% each; VA at 10%, and the remaining antimicrobials showed a susceptibilities of less than 10%.

3.2.2 Antimicrobial Susceptibilities of *L. rhamnosus, L. sakei* , and Unidentified LAB.

Table 5 shows the result of the antimicrobial susceptibilities of *L. rhamnosus, L. sakei* , and unidentified LABs.

Table 5. Distribution of resistant, intermediate and susceptible antimicrobials of *L. rhamnosus, L. sakei* and unidentified LAB isolated from Kimchi

Antibiotics	Nos. of isolates (%)								
	L. rhamnosus(n=7)			*L. sakei*(n=6)			Unidentified(n=40)		
	R	I	S	R	I	S	R	I	S
Ampicillin	-	1(14.3)	6(85.7)	-	2(33.3)	4(66.7)	14(35.0)	3(7.5)	23(57.5)
Streptomycin	-	-	7(100)	-	1(16.7)	5(83.3)	98(95.0)	-	2(5.0)
Sulfamethoxazole/ Trimethoprim	7(100)	-	-	6(100)	-	-	29(72.5)	3(7.5)	8(20.0)
Vancomycin	7(100)	-	-	5(83.3)	1(16.7)	-	36(90.0)	-	4(10.0)
Gentamicin	6(85.7)	-	1(14.3)	2(33.3)	-	4(66.7)	29(72.5)	-	11(27.5)
Tetracycline	-	-	1(100)	-	-	6(100)	12(30.0)	5(12.5)	23(57.5)
Oxacillin	1(14.3)	3(42.9)	3(42.9)	6(100)	-	-	29(72.5)	6(15.0)	5(12.5)
Norfloxacin	-	4(57.1)	3(42.9)	6(100)	-	-	35(87.5)	-	5(12.5)
Nalidixic acid	7(100)	-	-	6(100)	-	-	39(97.5)	-	1(2.5)
Erythromycin	1(14.3)	-	6(85.7)	-	-	6(100)	13(32.5)	-	27(67.5)
Cephalothin	-	1(14.3)	6(85.7)	-	-	6(100)	15(37.5)	8(20.0)	17(42.5)
Clindamycin	-	-	7(100)	-	-	6(100)	14(35.0)	6(15.0)	20(50.0)
Rifampicin		1(14.3)	6(85.7)	-	4(66.7)	2(33.3)	12(30.0)	8(20.0)	20(50.0)
Cefotetan	7(100)	-	-	6(100)	-	-	39(97.5)	-	1(2.5)
Chloramphenicol	-	-	7(100)	-	-	6(100)	12(30.0)	1(2.5)	27(67.5)
Penicillin	-	-	7(100)	-	3(50)	3(50)	12(30.0)	14(35)	14(35.0)
Imipenem	-	-	7(100)	-	-	6(100)	12(30.0)	-	28(70.0)
Ciprofloxacin	-	-	7(100)	6(100)	-	-	33(82.5)	1(2.5)	6(15.0)

R : resistant, I : intermediate, S : susceptible

Antibiotic susceptibilities for 7 strains of *L. rhamnosus* showed resistance to all of SXT, VA, NA and CTT; GM showed resistance of 85.7%; OX and E showed resistances of 14.3% each; and all other antibacterial agents showed no resistances.

In the case of intermediate resistance to antimicrobial agents, NOR was the highest at 57.1%; OX was at 42.9%; AM, CF and RA were at 14.3% each; and the rest of the antimicrobials had no intermediate resistances.

In the case of antimicrobial susceptibilities, TE, CC, CM, O, IMP, and CIP all showed susceptibilities;

AM, E, CF, and RA had the susceptibilities of 85.7% each; and OX and NOR each had the susceptibilities of 42.9% each; and GM had a susceptibility of 14.3%; while there were no susceptibilities to the rest of the antimicrobial agents.

In the case of antibiotic susceptibilities for the *L. sakei* 6 strains, SXT, OX, NOR, NA, CTT and CIP all showed resistances; intermediate resistance was the highest with RA at 66.7%; P of 50%, AM of 33.3%, S and VA of 16.7% each showed intermediate resistance; and the rest of the antimicrobials did not show any of intermediate resistances.

In the case of antimicrobial susceptibilities, TE, E, CF, CC, RA, P and IMP all showed susceptibilities; S showed susceptibility of 83.3%; AM and GM of 66.7% each; P of 50%; RA of 33.3%; but the rest of the antibacterial agents did not show any of susceptibilities.

3.2.3 Antibiotic Multi-Drug Resistances of *L. plantarum* Isolated From Kimchi.

The antibiotic multi-drug resistances in *L. plantarum* isolated from Kimchi are shown in Table 6.

Table 6. Multiple antimicrobial resistance patterns in *L. plantarum* isolated from Kimchi.

Class	Multiple antimicrobial resistance patterns	Nos. of isolates	Subtotal(%)
5	SXT-GM-NOR-NA-CIP	1	2(3.8)
	VA-NOR-NA-CTT-CIP	1	
6	S-SXT-VA-TE-NA-CTT	1	3(5.7)
	S-VA-NOR-NA-CTT-CIP	2	
7	S-GM-OX-NOR-NA-CTT-CIP	1	18(33.9)
	S-SXT-OX-NA-E-RA-CTT	1	
	S-VA-GM-NOR-NA-CTT-CIP	12	
	S-VA-GM-OX-NOR-NA-CTT	1	
	S-VA-OX-NOR-NA-CTT-CIP	2	
	VA-GM-OX-NOR-NA-CTT-CIP	1	
8	S-SXT-VA-GM-NOR-NA-CTT-CIP	1	25(48.2)
	S-VA-GM-NOR-NA-CC-CTT-CIP	1	
	S-VA-GM-NOR-NA-E-CTT-CIP	2	
	S-VA-GM-OX-NOR-NA-CTT-CIP	20	
	S-VA-OX-NOR-NA-CF-CTT-CIP	1	
9	S-SXT-VA-GM-OX-NOR-NA-CTT-CIP	4	4(7.5)
17	AM-S-SXT-VA-GM-OX-NOR-NA-E-CF-CC-RA-CTT-CM-P-IMP-CIP	1	1(1.9)
	Total	53	53

AM:ampicillin, S: streptomycin, SXT: sulfamethoxazole/trimethoprim, VA: vancomycin, GM: gentamicin, TE: tetracycline, OX: oxcillin, NOR: norfloxacin, NA: nalidixic acid, E: erythromycin, CF: cephalothin, CC: colistin, RA: rifampicin, CTT: cefotetan, C: chloramphenicol, P: penicillin, IMP: imipenem, CIP: ciprofloxacin.

For the multi-drug resistance of the 53 strains of *L. plantarum*, among the 53 antibiotic resistance strains, 8-drug resistance was the highest with 25 strains (47.2%); 7-drug resistance to 18 strains (33.9%); 9-drug resistance to 4 strains (7.5%); 6-drug resistance to 3 strains (5.7%); 5-drug resistance to 2 strains (3.8%); and 17-drug resistance to 1 strain (1.9%) followed.

Out of them, resistance to S-VA-GM-OX-NOR-NA-CTT-CIP were the highest with 20 strains (37.7%);

there were 12 strains (22.6%) resistant to S-VA-GM-NOR-NA-CTT-CIP; and 4 strains (7.5%) resistant to S-SXT-VA-GM-OX-NOR-NA-CTT-CIP. And resistances to S-VA-NOR-NA-CTT-CIP, resistances to S-VA-OX-NOR-NA-CTT-CIP, and resistance tos S-VA- GM-NOR-NA-E-CTT-CIP were for 2 strains (3.8%), in the order.

And were there shown the resistances of 1 strain to SXT-GM-NOR-NA-CIP; the resistances to VA-NOR-NA-CTT-CIP; the resistances to S-SXT-VA-TE-NA-CTT; the resistances to S-GM-OX-NOR-NA-CTT-CIP; the resistances to S-SXT-OX-NA-E-RA-CTT; the resistances to S-VA-GM-NOR-NA-CTT-CIP; the resistances to VA-GM-OX-NOR- NA-CTT-CIP; the resistances to S-SXT-VA-GM-NOR-NA-CTT-CIP; the resistances to S-VA-GM-NOR-NA-CF-CTT-CIP; and the resistances to AM-S-SXT-VA-GM-OX-NOR-NA-E-CF-CC-RA-CTT-CM-P-IMP-CIP .

3.2.4 Antibiotic Multi-Drug Resistances in *Ent. faecium* isolated From Kimchi. Figure 7 shows the antibiotic multi-drug resistances in *Ent. faecium* isolated from Kimchi.

Table 7. Multiple antimicrobial resistance patterns in *Ent. faecium* isolated from Kimchi

Class	Multiple resistance patterns	Nos. of isolates	Subtotal(%)
4	SXT-OX-NA-E-RA	1	1(3.7)
6	S-SXT-OX-NA-E-CTT	1	1(3.7)
7	S-SXT-GM-OX-NA-E-CTT	1	4(14.8)
	S-SXT-OX-NA-E-RA-CTT	3	
8	S-SXT-GM-OX-NA-E-CC-CTT	4	6(22.2)
	S-SXT-GM-OX-NOR-NA-E-CTT	1	
	SXT-GM-OX-NA-E-CC-RA-CTT	1	
9	S-SXT-GM-OX-NA-E-CC-RA-CTT	5	9(33.3)
	S-SXT-GM-OX-NA-E-CF-CC-CTT	3	
	S-SXT-GM-OX-NA-E-CF-CC-RA	1	
10	S-SXT-GM-OX-NA-E-CF-CC-RA-CTT	2	2(7.4)
11	AM-S-SXT-GM-OX-NA-E-CF-CC-RA-CTT	1	4(14.8)
	S-SXT-GM-OX-NOR-NA-E-CF-CC-RA-CTT	3	
	Total	27	27

AM: ampicillin, S: streptomycin, SXT: sulfamethoxazole/trimethoprim, VA: vancomycin, GM: gentamicin, TE: tetracycline, OX: oxcillin, NOR: norfloxacin, NA: nalidixic acid, E: erythromycin, CF: cephalothin, CC: clindamycin, RA: rifampicin, CTT: cefotetan, C: chloramphenicol, P: penicillin, IMP: imipenem, CIP: ciprofloxacin.

For the multi-drug resistance to the 27 strains of *Ent. Faecium*, out of the 27 antibiotic resistance strains, 9-drug resistance was the highest with 9 strains (33.3%)); 8-drug resistance to 6 strains (22.2%); 7-drug and 11-drug resistances to 4 strains (14.8%); and 4-drug and 6-drug resistances to 1 strain (1.9%).

Out of the patterns of multi-drug resistances, resistances to S-VA-GM-OX-NOR-NA-CTT-CIP were the highest with 5 strains (18.5%); there were 4 strains (14.8%) resistant to S-SXT-GM-OX-NA-E-CC-CTT; and resistances to S-SXT-OX-NA- E-RA-CTT; resistances to S-SXT-GM-OX-NA-E-CF-CC-CTT; and resistances to S-SXT-GM-OX-NOR-NA-E- CF-CC-RA-CTT were for 3 strains (18.5%).

There shown were 2 strains (7.4%) resistant to S-SXT-GM-OX-NA-E-CF-CC-RA-CTT. And were there shown the resistances of 1 strain to SXT-OX-NA-E-RA, the resistances to S-SXT-OX-NA-E-CTT; the resistances to S-SXT-GM-OX-NA-E-CTT; the resistances S-SXT-GM-OX-NOR-NA-E-CTT; the resistances to SXT-GM-OX-NA-E-CC-RA-CTT; the resistances to S-SXT- GM-OX-NA-E-CF-CC-RA,; and the resistances to AM-S-SXT-GM-OX-NA-E-CF-CC-RA-CTT .

4. Discussion

In 1994, the World Health Organization considered that LAB would be the most important immune defense mechanism for the next generation when the commonly prescribed antibiotics were rendered obsolete by antibiotic resistance. The outbreak of antibiotic resistance has opened the door for a new concept of LAB in the medical field.

It has been reported that *L. rhamnosus* exhibits antagonistic activity against pathogens, H_2O_2 generating ability, generation of organic acids and lactic acid, antioxidant and anti-inflammatory activities, and resistance to antibacterial agents [18].

L. sakei showed antibacterial activity and H_2O_2 generating ability against Listeria monocytogenes, Staphylococcus aureus, and Pseudomonas aeruginosa [19].

It has been reported that *Lactobacillus salivarius* exhibits high antibacterial activity, acidic pH-resistant lysozyme, and H_2O_2 generating ability [20]..

L. plantarum showed significant generating abilities in antifungal, antibacterial activity, organic acids, and H_2O_2 for yeasts (Rhodotorula glutinis and Candida pelliculosa), fungi (Penicillium digitatum, Aspergillus niger, Fusarium oxysporum, and Rhizopus oryzae), and pathogenic bacteria (Listeria monocytogenes and Salmonella enterica) [21].

Lactic acid bacteria (LAB) which generate H_2O_2 are involved in non-specific antibacterial action of the host under anaerobic or slightly anaerobic conditions.

In this study, we found that 6 strains from 27 strains *of Ent. Faecium*; 25 strains from 53 strains of *L. plantarum*; 1 strain from 7 strains of *L. rhamnosus*; 3 strains from 6 strains of *L. sakei*; 1 strain from 1 strain of *St. thermophilus*; and 7 strains from unidentified 40 strains, separated from Kimchi, produced H_2O.

When antibiotics are administered to long-term inpatients for a long period of time, beneficial LAB as well as harmful microorganisms present in the human body are killed, resulting in diarrhea, vaginitis and atopic dermatitis due to the imbalance of microorganisms.

We studied antibiotic resistance and mult-drug resistance in order to determine whether long-term inpatients can use them as an antibiotic substitute or an antibiotic adjuvant.

The results are as follows. In the case of antibiotic susceptibility of 27 strains of *Ent. Faecium*, SXT, OX, NA, and E were all resistant; and resistance to S and CTT was 92.6%, GM 81.5%, CC 74.1%, CTT 63.0%, CF 37.0%, and AM 3.7%, respectively; In the case of antibiotic susceptibility to 53 strains of *L. plantarum*, all NAs showed resistances. The resistances were 98.1% for CTT, 96.2% for NOR and CIP, 84.3% for VA and S, 84.9% for GM, 60.4% for OX and 17.0% for SXT.

The antibiotic susceptibility of one strain of *St.* thermophilus was resistant to NA, CC, RA, CTT, CM and P. Antibiotic susceptibility to 7 strains of *L. rhamnosus* showed resistance to all of SXT, VA, NA and CTT; GM showed resistance of 85.7%, OX and E showed resistance of 14.3% each.

In the case of antibiotic susceptibility for the *L. sakei* 6 strains, SXT, OX, NOR, NA, CTT and CIP all showed resistance

For the multi-drug resistance to the 27 strains of *Ent. Faecium*, among the 27 antibiotic resistance strains, 9-drug resistance was the highest with 9 strains (33.3%)); 8-drug resistance to 6 strains (22.2%); 7-drug and 11-drug resistances to 4 strains (14.8%); 4-drug and 6-drug resistances to 1 strain (3.7%).

Among the patterns of Multi-Drug Resistances, resistance to S-VA-GM-OX-NOR-NA-CTT-CIP was the highest with 5 strains (18.5%); there were 4 strains (14.8%) resistant to S-SXT-GM-OX-NA-E-CC-CTT, and resistance to S-SXT-OX-NA- E-RA-CTT, resistance to S-SXT-GM-OX-NA-E-CF-CC-CTT, and resistance to S-SXT-GM-OX-NOR-NA-E- CF-CC-RA-CTT were for 3 strains (11.1%).

There were 2 strains (7.4%) resistant to S-SXT-GM-OX-NA-E-CF-CC-RA-CTT. The resistances to SXT-OX-NA-E-RA, the resistances to S-SXT-OX-NA-E-CTT, the resistances to S-SXT-GM-OX-NA-E-CTT, the resistances S-SXT-GM-OX-NOR-NA-E-CTT, the resistances to SXT-GM-OX-NA-E-CC-RA-CTT, the resistances to S-SXT- GM-OX-NA-E-CF-CC-RA, and the resistances to AM-S-SXT-GM-OX-NA-E-CF-CC-RA-CTT were for 1 strain.

For the multi-drug resistance to the 53 strains of *L. plantarum,* among the 53 antibiotic resistance strains, 8-drug resistance was the highest with 25 strains (47.2%); 7-drug resistance to 18 strains (33.9%); 9-drug resistance to 4strains (7.5%); 6-drug resistance to 3 strains (5.7%); 5-drug resistance to 2 strains (3.8%); and 17-drug resistance to 1 strain (1.9%) followed.

Out of them, resistance to S-VA-GM-OX-NOR-NA-CTT-CIP was the highest with 20 strains (37.7%); there were 12 strains (22.6%) resistant to S-VA-GM-NOR-NA-CTT-CIP and 4 strains (7.5%) resistant to S-SXT-VA-GM-OX-NOR-NA-CTT-CIP. And resistance to S-VA-NOR-NA-CTT-CIP, resistance to S-VA-OX-NOR-NA-CTT-CIP, and resistance to S-VA- GM-NOR-NA-E-CTT-CIP were for 2 strains (3.8%), in the order.

Out of the resistances to SXT-GM-NOR-NA-CIP, the resistances to VA-NOR-NA-CTT-CIP, the resistances to S-SXT-VA-TE-NA-CTT, the resistances to S-GM-OX-NOR-NA-CTT-CIP, the resistances to S-SXT-OX-NA-E-RA-CTT, the resistances to S-VA-GM-NOR-NA-CTT-CIP, the resistances to VA-GM-OX-NOR- NA-CTT-CIP, the resistances to S-SXT-VA-GM-NOR-NA-CTT-CIP, the resistances to S-VA-GM-NOR-NA-CF-CTT-CIP, and the resistances to AM-S-SXT-VA-GM-OX-NOR-NA-E-CF-CC-RA-CTT-CM-P-IMP-CIP were for 1 strain.

Therefore, we demonstrated the antibacterial activity of LAB in previous studies and demonstrated that LAB has H_2O_2 generating ability and resistance to antibiotics in this study, and we proved that these can serve as antibiotic substitutes or antibiotic adjuvants for long-term inpatients.

However, since this is the result of an In Vitro study, it seems that more In Vivo studies are needed.

In addition, LAB is rich in antimicrobial resistance genes (ARGs), but it is not dangerous because it does not have a mobile genetic element (MEG). However, it was shown that the LAB has a high possibility of metastasis of antimicrobial resistance genes (ARGs) in the intestinal microbial population [22]

When we plan to develop commercial LAB strains, we should always take care.

5. Conclusion

Currently, LAB as a commercial strain is widely used in vaginitis treatments, health functional foods, and atopic dermatitis treatments.

Researches on LAB, as a substitute for antibiotics or as an adjuvant that can be used together with antibiotics for long-term inpatients, are still at an insignificant level.

The use of LAB in long-term hospitalized patients is thought to be effective in reducing troubles such as the intestines, skin, and female vagina.

In our study*, Ent. Faecium*, *L. plantarum*, *L. rhamnosus* , *L. sakei* , and *St. thermophilus,* shown to have anantibacterial activity in previous studies on LAB and shown to have and H_2O_2 generating ability, antibiotic resistance and multi-drug resistance in this study, are expected to be able to play an excellent role for long-term inpatients to use as an alternative to antibiotics and to deal with emerging antibiotic resistance.

Acknowledgement

Conflict of interest: The authors declare that there are no conflicts of interest

References

[1] K. Dong, J. Pu, J. Yang, G. Zhou, X. Ji, Z. Kang, J. Li, M. Yuan, X. Ning, Z. Zhang, X. Ma, Y. Cheng, H. Li, Q. Ma, L. Zhao, W. Lei, B. Sun and J. Xu, "The species-level microbiota of healthy eyes revealed by the integration of metataxonomics with culturomics and genome analysis", Front Microbiol 13, 950591, 2022
DOI : https://doi.org/10.3389/fmicb.2022.950591

[2] E. Månsson, S. Tevell, Å. Nilsdotter-Augustinsson, T. B. Johannesen, M. Sundqvist, M. Stegger and B. Söderquist, "Methicillin-Resistant *Staphylococcus epidermidis* Lineages in the Nasal and Skin Microbiota of Patients Planned for Arthroplasty Surgery", Microorganisms 9, no. 2, 2021.
DOI : https://doi.org/10.3390/microorganisms9020265

[3] Ö. Akgül, G. Söyletir and N. Ülger Toprak, "antimicrobial susceptibility of pathogenic gram-positive anaerobic cocci: Data of a university hospital in turkey", Mikrobiyol Bul 54, no. 3, 404-417, 2020
DOI : https://doi.org/10.5578/mb.69556

[4]. S. B. Orchanian, J. M. Gauglitz, S. Wandro, K. C. Weldon, M. Doty, K. Stillwell, S. Hansen, L. Jiang, F. Vargas, K. E. Rhee, J. C. Lumeng, P. C. Dorrestein, R. Knight, J. H. Kim, S. J. Song and A. D. Swafford, "Multiomic analyses of nascent preterm infant microbiomes differentiation suggest opportunities for targeted intervention", Adv Biol (Weinh) 6, no. 8, e2101313, 2022
DOI : https://doi.org/10.1002/adbi.202101313

[5] Y. Xu, O. Milburn, T. Beiersdorfer, L. Du, H. Akinbi and D. B. Haslam, "Antibiotic exposure prevents acquisition of beneficial metabolic functions in the preterm infant gut microbiome", Microbiome 10, no. 1, 103, 2022
DOI : https://doi.org/10.1186/s40168-022-01300-4

[6] P. G. Pappas, M. S. Lionakis, M. C. Arendrup, L. Ostrosky-Zeichner and B. J. Kullberg, "Invasive candidiasis", Nat Rev Dis Primers 4, 18026, 2018.
DOI : https://doi.org/10.1186/s40168-022-01300-4

[7] C. E, M. MT, A. AG, F. R, G. H, J. H, J. SZ, B. SL, D. S, H. AU, B. D, D. L, P. JS and M. L, "Antimicrobial and inflammatory properties of south african clinical *lactobacillus* isolates and vaginal probiotics", Scientific reports 9, no. 1, 2019.
DOI : https://doi.org/10.1038/s41598-018-38253-4

[8] B. JM, D. E, B. F, B. G, D. C and C. JM, "Efficacy and safety of vaginally administered lyophilized *lactobacillus crispatus ip 174178* in the prevention of bacterial vaginosis recurrence", Journal of gynecology obstetrics and human reproduction 47, no. 2, 2018.
DOI : https://doi.org/10.1016/j.jogoh.2017.11.005

[9] Y. S. Yuk, Y. k. Lee and G.-Y. Kim, "Antagonistic inhibitory effects of probiotics against pathogenic microorganisms *in vitro*", Journal of the Korea Academia-Industrial cooperation Society 20, no. 12, 110-116, 2019.
DOI : https://doi.org/10.5762/KAIS.2019.20.12.110

[10] J. S. Kim, Y. S. Yuk and G. Y. Kim, "Inhibition effect on pathogenic microbes and antimicrobial resistance of probiotics", Korean Journal of Clinical Laboratory Science 51, no. 3, 294-300, 2019.
DOI : https://doi.org/10.15324/kjcls.2019.51.3.294

[11] Y. S. Yuk, Y. K. Lee and G.-Y. Kim, "Antagonistic effects of *lactobacillus plantarum* on *candida albicans* in me-180 cervical carcinoma cell culture", Jundishapur Journal of Microbiology 13, no. 11, 2020.
DOI : https://doi.org/10.5812/jjm.112449

[12] L. K. Rabe and S. L. Hillier, "Optimization of media for detection of hydrogen peroxide production by *lactobacillus* species", J Clin Microbiol 41, no. 7, 3260-3264, 2003.
DOI : https://doi.org/10.1128/JCM.41.7.3260-3264.2003

[13] D.-K. Kang, H. K. Oh, J.-S. Ham, J. G. Kim, C. H. Yoon, Y. T. Ahn and H. U. Kim, "Identification and characterization of hydrogen peroxide-generating *lactobacillus fermentum cs12-1*", Asian-Australasian Journal of Animal Sciences 18, no. 1, 90-95, 2005
DOI : https://doi.org/10.5713/ajas.2005.90

[14] R. M. Humphries, J. Ambler, S. L. Mitchell, M. Castanheira, T. Dingle, J. A. Hindler, L. Koeth, K. Sei, D. Hardy, B. Zimmer, S. Butler-Wu, J. D. Bard, B. Brasso, R. Shawar, T. Dingle, R. Humphries, K. Sei, L. Koeth and C. S. Kraft,

"Clsi methods development and standardization working group best practices for evaluation of antimicrobial susceptibility tests", Journal of Clinical Microbiology 56, no. 4, 2018.
DOI : https://doi.org/10.1128/JCM.01934-17

[15] W. H. Organization(WHO), "Manual for the laboratory identification and antimicrobial susceptibility testing of bacterial pathogens of public health importance in the developing world," 2003.

[16] J. Han, D. Chen, S. Li, X. Li and W.-W. Zhou, "Antibiotic susceptibility of potentially probiotic *lactobacillus* strains", Italian Journal of Food Science 27, no. 3, 282-289, 2015.
DOI : https://www.proquest.com/docview/1721465255/96D90DE2397B4A47PQ/1?accountid=10536

[17] R. Coppola, M. Succi, P. Tremonte, A. Reale, G. Salzano and E. Sorrentino, "Antibiotic susceptibility of *lactobacillus rhamnosus* strains isolated from parmigiano reggiano cheese", Le Lait 85, no. 3, 193-204, 2005.
DOI : https://doi.org/10.1051/lait:2005007

[18] A. Pino, A. Vaccalluzzo, C. Caggia, S. Balzaretti, L. Vanella, V. Sorrenti, A. Ronkainen, R. Satokari and C. L. Randazzo, "*Lacticaseibacillus rhamnosus CA15 (DSM 33960)* as a Candidate Probiotic Strain for Human Health", Nutrients 14, no. 22, 2022
DOI : https://doi.org/10.3390/nu14224902

[19] E. Dincer and M. Kivanc, "Evaluation of metabolic activities and probiotic characteristics of two *latilactobacillus sakei* strains isolated from pastırma", World J Microbiol Biotechnol 38, no. 12, 237, 2022.
DOI : https://doi.org/10.1007/s11274-022-03431-0

[20] Y. Yang, X. Song, Z. Xiong, Y. Xia, G. Wang and L. Ai, "Complete genome sequence of *lactobacillus salivarius ar809,* a probiotic strain with oropharyngeal tract resistance and adhesion to the oral epithelial cells", Curr Microbiol 79, no. 9, 280, 2022
DOI : https://doi.org/10.1007/s00284-022-02963-w

[21] H. Abouloifa, Y. Rokni, I. Hasnaoui, R. Bellaouchi, S. Gaamouche, N. Ghabbour, S. Karboune, R. Ben Salah, M. Brasca, G. D'hallewin, E. Saalaoui and A. Asehraou, "Characterization of antimicrobial compounds obtained from the potential probiotic *lactiplantibacillus plantarum s61* and their application as a biopreservative agent", Braz J Microbiol 53, no. 3, 1501-1513, 2022
DOI : https://doi.org/10.1007/s42770-022-00791-5

[22] V. Rozman, P. Mohar Lorbeg, P. Treven, T. Accetto, M. Golob, I. Zdovc and B. Bogovič Matijašić, "Lactic acid bacteria and *bifidobacteria* deliberately introduced into the agro-food chain do not significantly increase the antimicrobial resistance gene pool", Gut Microbes 14, no. 1, 2127438, 2022.
DOI : https://doi.org/10.1080/19490976.2022.2127438