Original Research

Journal of Advanced Veterinary Research
(2024) Volume 14, Issue 3, 481-485

Antibacterial activity of probiotic bacteria against pathogenic bacteria isolated from different chicken organs

Aya M. Soliman*, Mohamed F. Ghaly, Eman Y. Elariny

Department of Botany and Microbiology, Faculty of Science, Zagazig University, Zagazig, 44519, Egypt.

ARTICLE INFO

Recieved: 17 November 2023

Accepted: 08 January 2024

***Correspondence:**

Corresponding author: Aya M. Soliman
E-mail address: aya.soliman2196@gmail.com

Keywords:

Antimicrobial agents, Bacterial infections
Multidrug resistance (MDR)
Lacto*Bacillus* bacteria (LAB)

ABSTRACT

Even though a wide variety of antimicrobial medications have been discovered and developed, multidrug resistance still poses a severe threat to public health and is continuously increasing mortality. Recently, a lot of research has focused on developing solutions to these problems. In this paper, we demonstrated how various antibiotics can be combined with antimicrobial substances like LAB and plant extracts to provide synergistic effects if each therapy concentrates on a different target or signalling pathway and acts via a different mechanism. MDR bacteria were isolated from chicken organs using standard methods, and they were confirmed using 16S RNA. In Al-Sharkia Governorate, Egypt, 100 different samples of chicken organs (including the breast, drumstick, liver, wings, skin, intestine, pins, giblets, heart, and legs) were taken at random from six different sites. *Bacillus* was the associated pathogenic bacteria obtained from the isolated chicken organs and was confirmed using molecular, biochemical, and microbiological techniques. The most efficient extracts that proved to have bacteriostatic and bactericidal properties against the highly resistant strains of pathogenic bacteria were found to be the *LactoBacillus plantarum* EMCC1027. The LAB applied in the study has excellent antibacterial properties and can be used as a safe substitute for antibiotics to inhibit the growth of the food-borne pathogens.

Introduction

One of the top global public health challenges is the prevention and management of multidrug-resistant organisms (MDROs), particularly in nations with inadequate health resources. (Byarugaba, 2004; Levy and Marshall, 2004; Mir and Zaidi, 2009). MDR organisms in the pollution might potentially worsen the existing serious threat of antimicrobial resistance (AMR), which is predicted to result in an annual 10 million mortality rate (Wassenaar, 2005; Vishnuraj *et al*., 2016; Mund *et al*., 2017; Lee *et al*., 2020)

The ability of a bacterium to resist the inhibitory or destructive effects of an antimicrobial to which it was not previously resistant is known as antibiotic resistance (Dehbanipour *et al*., 2016; Motse *et al*., 2019; WHO, 2019). This adaptive process is mostly brought on by the bacterial enzymatic degradation of antibiotics, the alteration of the antibiotic target, antibiotic overuse, and the change in membrane permeability, and alternative metabolic pathways (Arsene *et al*., 2022)

MDR species can be found in people, animals, plants, food, water, soil, and air, in addition to hospitals and other healthcare facilities (Nagaraj and Kwang-Hyun, 2022). Foodborne disease, which is on the rise globally and is especially prevalent in people who eat meals away from home because of the uncontrolled hygienic preparation of these types of food, was defined by the WHO as an infectious or toxic disease that is contracted through the consumption of contaminated food (Noori and Alwan, 2016)

In order to combat antibiotic resistance, it is necessary to look for alternative, effective antimicrobial drugs and more focused treatment methods. Using various antimicrobial drugs in combination to provide synergistic effects is a different way that is currently being used or is undergoing testing (Kaur, 2016). Combination therapy is a desirable and optional treatment since it lowers the probability of developing cross-resistance and provides possible adjuvant targets for distinct signalling pathways (Bozic *et al*., 2013).

The use of probiotics as alternative biological antimicrobial agents is effective in reducing the number of pathogenic bacteria that are resistant to common antibiotics. Probiotics are living microorganisms that, when consumed in sufficient amounts, have a positive impact on the health of the host (Hill *et al*., 2014).

The most popular probiotics are Lactobacilli bacteria, which are regarded as beneficial gut microflora (Shokryazdan *et al*., 2014). They are gram-positive, non-spore-forming bacteria found mainly in dairy products (Rabiul *et al*., 2020).The synthesis of antimicrobial compounds by Lactobacilli, including lactic acid, hydrogen peroxide, and bacteriocin, is what gives them their preserving properties. In the food business, bacteriocins are primarily employed to stop food spoilage and food-borne illnesses. Bacteriocins are widely regarded as safe for usage and have a number of desirable properties, including nontoxicity, inactivation by digestive tract-related proteases, genetic engineering, and being used as natural food preservatives (Galvez *et al*., 2007; Perez *et al*., 2014; Usman *et al*., 2022).The combination's synergism lowers the minimum inhibitory concentrations (MICs) of these drugs, as well as its financial burden and sensory impact (Reda *et al*., 2017). So, the aim of this study was to explore the antibacterial capabilities of probiotics combined with traditional antibiotics to treat a variety of multiresistant pathogenic bacteria.

Materials and methods

Collection of samples

In Al-Sharkia, 100 (one hundred) different chicken samples were ran-

ISSN: 2090-6277/2090-6269/

domly selected from six different places. Chicken organs (breast, drumstick, liver, wings, skin, intestinal, pins, giblets, heart, and legs) were collected from different locations in Al-Sharkia governorate collected from supermarkets and retail markets during the period from November 8, 2021, until May 24, 2022.

Chicken samples weighing 100 g were obtained and placed in sterile, dry, and clean polythene bags before being delivered to the lab for microbiological analysis.

Sample culturing

The samples were aseptically cut into tiny, thin pieces using a sterile knife. The analytical components were homogenized in different sterile plastic bags before being combined with 250 mL of distilled water to create the stock. In the serial dilution experiment, each produced sample was serially diluted up to fivefold (10–5) using 1 mL of stock homogenate and 9 mL of sterile distilled water. This was carried out to obtain a separate colony. Pre-serially diluted samples (0.1 mL) were applied using the spread plate method on pre-made solidified nutrient agar plates. The mixture was given five (5) minutes to set completely before inoculation; plates were placed in an incubator at 37°C for 24 hours (Oluwatobi *et al*., 2021).

Antibiotic susceptibility test by disc diffusion method

The isolates were tested for antibiotic sensitivity using Kirby Bauer's disc diffusion method on nutrient Agar (NA) using easily available commercial antibiotic discs. A sterile cotton brush soaked in bacteria suspension was used to do a grass culture on the surface of a NA plate. Antibiotic discs that were easily found on the market were applied to the surfaces of the cultured plates. The plates were incubated at 37°C for 16–18 hours. Each antimicrobial drug's zones of inhibition were assessed after incubation, and the results were compared to the NCCLS chart (Oluwatobi *et al*., 2021).

Determination of Minimum Inhibitory Concentrations (MIC) and Minimum Bactericidal Concentration (MBC)

The MIC is the lowest concentration of an antibacterial agent that completely stops bacterial growth. The MIC of the different extracts was determined using the broth microdilution technique. In a nutshell, a 96-well U-bottom microplate was filled with 100 µL of bacterial culture in the first row (columns 1–10) (Manga *et al*., 2021; Konstantinovitch *et al*., 2022).

Each antibiotic dilution was added to them in 100 µL. Then, as a negative control, 100 µL of sterile broth devoid of culture was added to the well of column 11. Then column 12's well received a 100 µL positive control of bacterial culture. The plates were then covered and incubated for 24 hours at 37°C. After incubation, MIC was regarded as the lowest concentration of the tested substance that prevented the bacteria from growing in an obvious way. MBCs were identified by subculturing the wells on plates devoid of growth (with concentrations ≥MIC). Agar plates that had been inoculated were incubated for 24 hours at 37°C. MBC was regarded as the lowest dose that prevented bacterial growth on agar (Mbarga *et al*., 2022).

Antimicrobial activity of Lactic Acid bacteria against indicator bacteria

Activation of Lactic acid strains

Four strains are used: *LactoBacillus acidophilus*, *LactoBacillus plantarum*, *LactoBacillus helveticus*, and *LactoBacillus rhamnosus*, were acquired from Cairo MIRCEN (Microbiological Resource Centre), Faculty of Agriculture, Ain Shams University, Cairo, Egypt. At 37°C for 24 hours, Biolife Italy's MRS broth (De Man *et al*., 1960) was used to activate all strains.

Primary Screening

The initial screening was done to determine how antimicrobial chemicals worked together. An agar well diffusion antimicrobial experiment was used to test the antibacterial activity of Lactic acid bacteria (Balouiri *et al*., 2016). In order to create active cultures, the pathogenic indicator bacterial strains were injected into a test tube containing 5 mL of nutrient broth and incubated at 37°C for 24 hours while being shaken at 180 rpm. Using sterile cotton swabs or a sterilized glass spreader, the whole surfaces of each nutrient agar plate were swabbed with the indicator bacteria (Lelise *et al*., 2014). Further characterization was done on the LAB isolates that exhibited the greatest zone of inhibition against indicator bacteria. These isolates were sub-cultured on MRS agar and preserved in a 15% tryptic soy broth glycerol solution for use in subsequent screening procedures, such as the extraction and characterization of antibacterial compounds (Girma and Aemiro, 2022).

Secondary screening

Preparation of cell-free supernatant

The procedures used to create the cell-free supernatants (CFSs) were somewhat modified from those used by Assefa (2022). LAB isolates were put into 5 mL of nutrient broth and incubated for 24 hours at 37°C. To obtain the supernatant, the broth culture was centrifuged at 10,000 rpm at 4°C for 20 minutes. Next, 1 M NaOH was used to adjust the supernatant's pH to 7 in order to remove the inhibitory effects of organic acid. According to the procedure adopted by Al-Allaf *et al*. (2009), the antimicrobial activity of CFS was tested.

Combination between the antibacterial activity of antibiotics and probiotic by disc diffusion method

Synergistic bacterial testing

Several antibiotics were alternately mixed with probiotics to test their synergistic antibacterial activity. This was done using the disc diffusion method, excluding at this point two antimicrobial agents—the selected antibiotic (at the MIC value) and LAB CSF, which were coated on sterile filter paper discs.

An antibiotic was combined and applied to the inoculated agar plates. The CSF from the LAB discs was added after doing this. The antimicrobial medication combination's zones of inhibition were estimated during an overnight incubation in accordance with the following description: Zones of combination treatment were classified as synergistic if they were greater than the zones of antibiotic, no effect if they were equal to the zones of antibiotic, and antagonistic if they were less than the zones of antibiotic (Shahabe *et al*., 2021).

Molecular identification

The active isolates were molecularly verified, by utilizing a forward primer for the 16S rRNA gene PCR (5'- AGAGTTTGATCMTGGCTCAG-3') and reverse primer (5'- TACGGYTACCTTGTTACGACTT-3') (Lagacé *et al*., 2004). Using 1.2% agarose gel electrophoresis, the amplicons' sizes were verified (Applichem, Germany, GmbH). 0.5 mg/ml ethidium bromide was used to stain (Sigma-Aldrich, MO, USA) and the identities were confirmed by automated DNA sequencing on an Applied Biosystems 3130 in both forward and reverse modes. (ABI, 3130, USA) employing a Bigdye Terminator ready for action V3.1 cycle sequencing kit (Perkin-Elmer/Applied Biosystems, Foster City, CA; Cat. No. 4336817). Using the Basic Local Alignment Search Tool (BLAST) available on the National Centre for Bio-

technology Information (NCBI) website (www.ncbi.nlm.nih.gov), the DNA sequences were compared to the GenBank sequences. MEGA11 was used to analyze nucleotide sequences (www.megasoftware.net).

Results

The results of the distribution percentage and number of bacteria isolated from each chicken organ indicated that the most isolated bacteria were collected from the breast (19.6%) and drumstick (16.1%). However, the minimum isolated were presented in the skin (3.6%) and legs (4.5%). The other sources of isolated samples showed an oscillatory distribution ($p<0.001$; Figure 1).

The chosen isolates' antibiotic susceptibility to 10 antibiotics from various categories revealed that the susceptibility of all isolated bacteria against all studied antibiotic drugs showed significant differences ($p<0.05$). In the same context, the isolated bacteria were more resistant to Amoxicillin, Cefepime, Cefuroxime, Cefatraixone, and Cefatoxime, while they were more susceptible to Ciprofloxacin, Cotrimoxazole, Vancomycin, and Tetracycline ($p<0.05$; Table 1).

Results of minimum inhibitory (MIC) and minimum bactericidal (MBC) concentrations (µg/ml) for drug choice against selected bacterial isolates clearly indicated that there was a significant difference ($p<0.05$) between isolated bacteria from DM3, FL8, and W6 with all studied drugs. Maximum values of MIC and MBC were recorded for FL8 and W6 isolated bacteria treated by Ciprofloxacin and Tetracycline and for DM3 and FL8 isolated bacteria treated by Amoxicillin and Ceftriaxone, respectively. Regarding MBC results, the maximum values were observed for W6 isolated bacteria treated by Ciprofloxacin and Tetracycline ($p<0.05$; Table 2).

There were significant differences ($p<0.05$) between inhibition zone diameters of selected MDR bacteria with all probiotic treatment, including *LactoBacillus plantarum* ($p>0.05$). The largest inhibition zone diameter was observed with W6 isolated bacteria treated by probiotic ($p<0.05$; Figure 2).

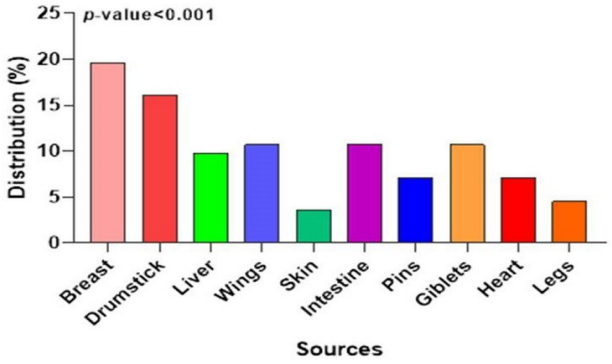

Fig. 1. Distribution percentage and number of bacteria isolated from each chicken organ.

The antibacterial effects of the treatment by *Lactobacillus plantarum* EMCC1027 at different incubation periods showed significant effects on the inhibition zone diameter of MDR bacteria maximized during the third and fifth days post treatment ($p<0.05$; Figure 3).

Table 1. Susceptibility test of different antibiotic drugs against selected bacterial isolates.

Antibiotics	Susceptible (S)		Intermediate (I)		Resistant (R)		*p*-value
	No.	%	No.	%	No.	%	
Ciprofloxacin (CIP)	11	57.9	5	26.3	3	15.8	0.00
Amoxicillin (AX)	4	21.1	0	0	15	78.9	<0.0001
Cefepime (FEP)	5	26.3	3	15.8	11	57.9	0.00
Cotrimoxazole (SXT)	10	52.6	1	5.3	8	42.1	0.01
Cefuroxime (CXM)	1	5.3	1	5.3	17	89.4	<0.0001
Vancomycin (VA)	13	68.4	3	15.8	3	15.8	0.00
Tetracycline (TE)	13	68.4	1	5.3	5	26.3	<0.0001
Gentamicin (CN)	6	31.6	3	15.8	10	52.6	0.05
ceftriaxone (CRO)	2	10.5	1	5.3	16	84.2	<0.0001
Cefatoxime (CTX)	5	26.3	2	10.5	12	63.2	0.00

Table 2. Minimum inhibitory (MIC) and minimum bactericidal (MBC) concentrations (µg/ml) for drugs choice against selected bacterial isolates.

Bacterial isolates code	Ciprofloxacin (CIP)		Amoxicillin (AX)		Tetracycline (TE)		Ceftriaxone (CRO)	
	MIC µg/ml	MBC µg/ml	MIC µg/ml	MBC µg/ml	MIC µg/ml	MBC µg/ml	MIC µg/ml	MBC µg/ml
DM 3	7.8^{b}	15.6^{b}	250^{a}	500^{a}	15.6^{b}	31.2^{c}	125^{b}	250^{b}
FL 8	62.5^{a}	125^{a}	125^{b}	250^{b}	62.5^{a}	125^{b}	250^{a}	500^{a}
W 6	62.5^{a}	125^{a}	125^{b}	250^{b}	62.5^{a}	250^{a}	125^{b}	250^{b}
p-value	0.00	<0.0001	0.00	0.00	0.00	<0.0001	<0.0001	<0.0001

[a,b]Means in the same column with different superscript letter following them are significantly different ($p < 0.05$)

Table 3. Combination effect of selected antibiotic and *Lactobacillus plantarum*

Bacterial isolates Code	Antibiotics alone (MIC)				*LactoBacillus planetarium*	Combination clove extract with *Lactobacillus plantarum*							
	CRO	AX	TE	CIP		CRO	Effect	AX	effect	TE	effect	CIP	effect
DM 3	18	10	6	19	23	20	S	10	N	10	S	13	A
FL 8	21	9	7	7	18	24	S	12	S	12	S	12	S
W 6	15	20	7	13	20	15	N	18	A	18	S	11	A
p-value	0.04	<0.001	0.68	<0.001	0.18	0.00		0.00		0.00		0.15	

S: Synergism; A: Antagonism; N: No effect

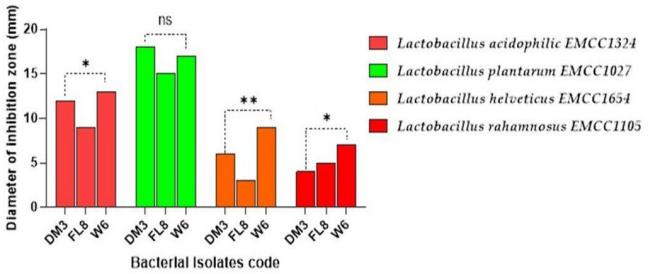

Fig. 2. Antibacterial activity of *LactoBacillus acidophilus* EMCC1324, *LactoBacillus plantarum* EMCC1027, *LactoBacillus helveticus* EMCC1654, and *LactoBacillus rhamnosus* EMCC1105 on selected MDR bacteria.

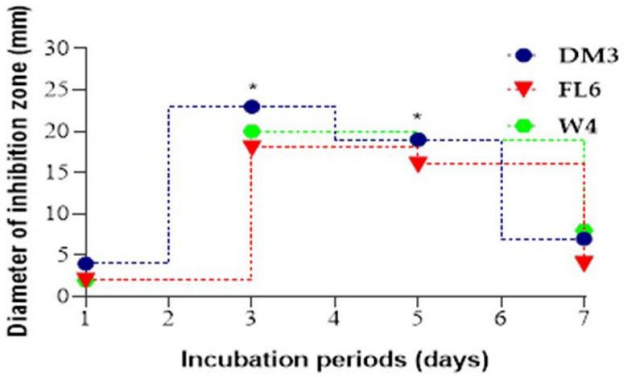

Fig. 3. Antibacterial effects of *Lactobacillus plantarum* EMCC1027 at different incubation periods against MDR bacteria. *p<0.0.

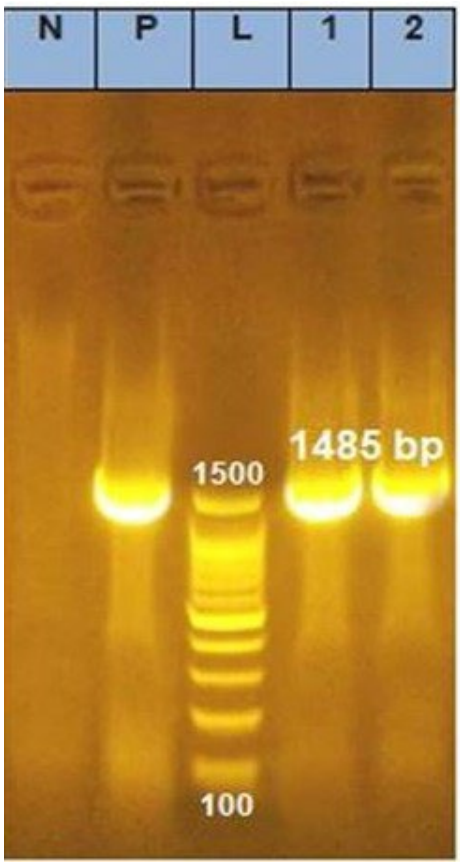

Fig. 4. Agarose gel electrophoresis for the amplified products of 16S RNA gene of *Bacillus* species (GenBank accession OQ557492).

The combination of antibiotics and *Lactobacillus plantarum* EMCC1027 bacteria against selected MDR bacteria had significant effects on the inhibition zone diameter except for the effects of tetracycline and probiotic alone, and the combination of ciprofloxacin and probiotic showed non-significant effects (p>0.05). The inhibition zone diameter minimized for DM3, FL8 and W6 isolated bacteria treated by tetracycline with non-significant differences (p>0.05; Table 3).

16S rRNA analysis for one type of bacteria using molecular techniques is one of the modern tools for the classification and identification of bacteria. After studying the morphology and physiology characters, molecular methods are applied for better taxonomical data. The name of the bacteria is *Bacillus firmicutes*.

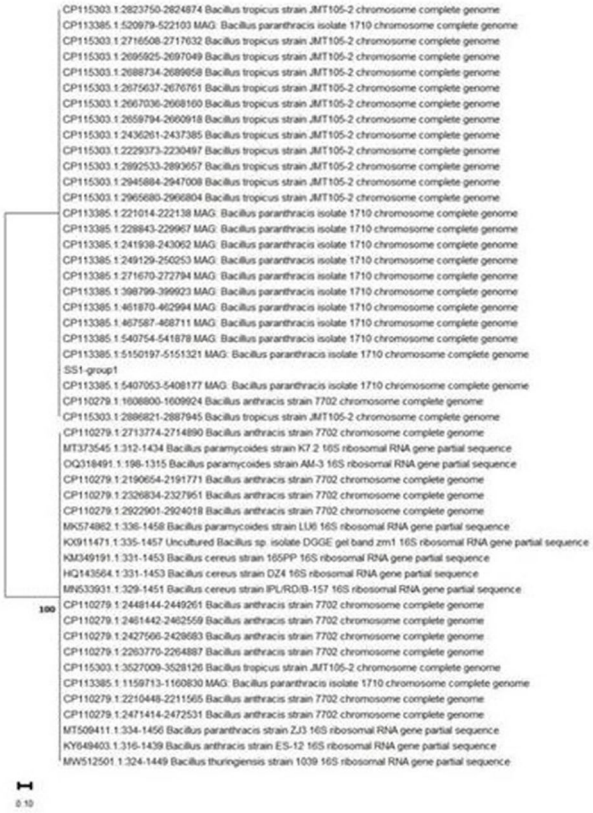

Fig. 5. *Bacillus firmicutes* strain MCCC 1A04098 16S ribosomal RNA, partial sequence. Gen bank number OQ557492.

The strain displayed a taxonomic correlation with the isolated strain based on the comparative analysis of the isolate *Bacillus* with the sequencing of the closest type species obtained by the NCBI BLAST method based on the trimmed and merged 16s rRNA sequencing analysed using gene bank nucleotide blast alignment tools. DNA sequencing of 1485 bp amplicons of the 16S rRNA gene confirmed the identification of *Bacillus* sp. (GenBank accession OQ557492).

Discussion

The goal of the current investigation was to identify the types and distribution of bacterial pathogens. The results in Table 1 indicate that breast has a high proportion of *Bacillus* occurrence; according to Public Health England (2009), the acceptable amount of *Bacillus* group bacteria in food is less than 103 cfu/g or ml. Food poisoning, however, may be brought on by dosages as low as 103 B. cereus cfu/g or m1 of food sample (Gilbert and Kramer, 1986; Stenfors *et al*., 2008) a pathogen that is common and can be found in several cuisines.

Minimum inhibitory (MIC) and minimum bactericidal (MBC) concentrations (µg/ml) chose drugs against selected bacterial isolates. In this study, commercially available and commonly used 10 antibiotics such as Amoxicillin, Gentamicin, Cefepime, Cotrimoxazole, Cefuroxime, Cefatraixone, and Cefatoxime, while they were more susceptible to Ciprofloxacin, Vancomycin and Tetracycline (Sigma, Saint Louis, MO, USA), were selected for antibacterial susceptibility testing. Antibiotics with a broad spectrum called gentamicin prevent the creation of new proteins. Overall disc diffusion assay findings showed that all isolated microorganisms were sensitive to Ofloxacin and Gentamicin but resistant to Amoxicillin and Cefotaxime, which was statistically significant (p <0.05) (Kiyomizu *et al*., 2009).

The MIC profiles indicate that the majority of the antimicrobial agents

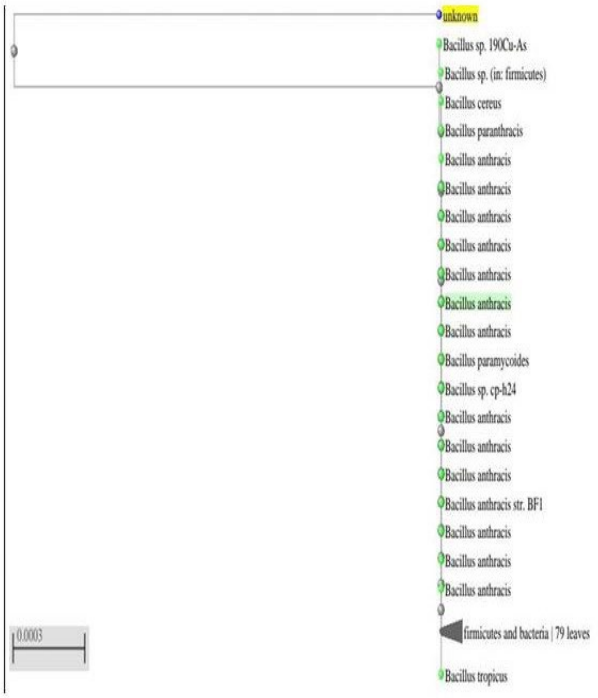

Fig. 6. Phytogenetic tree of *Bacillus firmicutes* (GenBank accession OQ557492.

estimated in the study only exhibited mild levels of resistance. However, a considerable level of resistance to all examined medications except Ciprofloxacin was detected.

Antibacterial effects of different plant extracts against selected most resistant bacterial isolates. During phytochemical screening, a variety of secondary metabolites, including alkaloids, polyphenols, flavonoids, anthraguinones, coumarins, saponins, tannins, triterpenes, and steroids, were found. Many compounds are active against harmful microorganisms (Jennifer, 2001; Erfan and Marouf, 2019).

The conventional agar disc diffusion technique was used to assess the methanolic extracts of the aforementioned plants' antibacterial properties. Antibacterial activity of *LactoBacillus acidophilus* EMCC1324, *LactoBacillus plantarum* EMCC1027, *LactoBacillus helveticus* EMCC1654, and *LactoBacillus rhamnosus* EMCC1105 on selected MDR bacteria.

The ability of *LactoBacillus acidophilus* and *LactoBacillus rhamnosus* to create beneficial substances during milk fermentation was found to have the greatest impact in a study by Griffiths *et al*. The efficient proteolytic system of *LactoBacillus plantarum* and *LactoBacillus rhamnosus*, which appears to be the most effective among LAB and has a significant impact on the generation of bioactive peptide , is what gives these bacteria their economic significance. *LactoBacillus helveticus*: the proteolytic system (Griffiths and Tellez, 2013).

The probiotic capabilities of *LactoBacillus plantarum* and *LactoBacillus rhamnosus* from pharmaceutical sachets revealed growth inhibitory capability against gram-positive as well as gram-negative bacterial strains, according to Monteagudo-Mera *et al*. (2012).

Conclusion

This study demonstrated that *LactoBacillus plantarum* EMCC1027 had excellent antimicrobial properties and can be used safely to replace antibiotics in inhibiting the growth of the food-borne pathogens.

Conflict of interest

The authors declare that they have no conflict of interest.

References

Al-Allaf, M.A.H., Al-Rawi, A.M.M., Al-Mola, A.T., 2009. Antimicrobial activity of lactic acid bacteria isolated from minced beef meat against some pathogenic bacteria. Iraqi J. Vet. Sci. 23, 3.

Arsene, M.M.J., Jorelle, A.B., Sarra, S., Viktorovna, P.I., Davares, A.K., Ingrid, N.K., Steve, A.A., Andreevna, S.L., Vyacheslavovna, Y.N., Carime, B.Z., 2021. Short review on the potential alternatives to antibiotics in the era of antibiotic resistance. J. Appli. Pharmac. Sci. 12, 29-40.

Assefa, E., 2022. Antimicrobial activity of lactic acid bacteria isolated from _ergo, Ethiopian traditional fermented milk, on some food borne pathogens, [M.S. thesis] Addis Ababa University.

Balouiri, M., Sadiki, M., Ibnsouda, S.K., 2016. Methods for _invitro_ evaluating antimicrobial activity: a review. J. Pharm. Anal. 2, 71-79.

Bozic, I., Reiter, J.G., Allen, B., Antal, T., Chatterjee, K., Shah, P., Moon, Y.S., Yaqubie, A., Kelly, N., Le, D.T., 2013. Evolutionary dynamicsof cancer in response to targeted combination therapy. Elife 2, e00747.

Byarugaba, D.K., 2004. A view on antimicrobial resistance in developing countries and responsible risk factors. Inter. J. Antimicrob. Agents 24, 105–110.

De Man, J.C., Rogosa, M., Sharpe, M.E., 1960. A medium for the cultivation of Lactobacilli. J. Appl. Bacterial. 23, 130-135.

Dehbanipour, R., Rastaghi, S., Sedighi, M., Maleki, N., Faghri, J., 2016. High prevalence of multi-drug-resistance uropathogenic Escherichia coli strains, Isfahan, Iran. J. Nat. Sci. Biol. Med. 7, 22-26.

Erfan, A.M., Marouf, S., 2019. Cinnamon oil downregulates virulence genes of poultry respiratory bacterial agents and revealed significant bacterial inhibition: An in vitro perspective. Vet. World 12, 1707-1715.

Galvez, A., Abriouel, H., Lopez, R.L., Ben, O.N., 2007. Bacteriocin-based strategies for food biopreservation, International J. Food Microbiol. 120, 51–70,

Gilbert, R.J., Kramer, J.M., 1986. *Bacillus* Cereus Food Poisoning, in Progress, in Food Safety: Proceedings of Symposium, eds D.C. Cliver and B.A. Cochrane (Madison, WI: Food Research Institute, University of Wisconsin-Madison) 10, 85–93.

Girma, A., Aemiro, A., 2022. Antibacterial activity of lactic acid bacteria isolated from fermented Ethiopian traditional dairy products against food spoilage and pathogenic bacterial strains. J Food Qual. 2022, 865-997.

Griffiths, M.W., Tellez, A.M., 2013. *LactoBacillus helveticus*: the proteolytic system. Front. Microbiol. 5, 4-30.

Hill, C., Guarner, F., Reid, G., Gibson, G.R., Merenstein, D.J., Pot, B., Morelli, L., Canani, R.B., Flint, H.J., Salminen, S., Calder, P.C., 2014. The International Scientific Association for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic. Nature Rev. Gastroenterol. Hepatol. 11, 506-514.

Jennifer, M., 2001. Determination of minimum inhibitory concentrations. J. Antimicrob. Chemother. 48, 5-16

Kaur, 2016. Novel strategies to combat antimicrobial resistance. J. Infect. Dis. Ther. 4, 292.

Kiyomizu, K., Yagi, T., Yoshida, H., Minami, R., Tanimura, A., Karasuno, T., Hiraoka, A., 2008. Fulminant septicemia of *Bacillus* cereus resistant to carbapenem in a patient with biphenotypic acute leukemia. J. Infect. Chemother. 14, 361-367.

Konstantinovitch, K.Y., Arsene, M.M.J., Aliya, M.V., Viktorovna, P.I., Elena, V.G., Azova, M.M., 2022. Assessment of Antimicrobial Activity of Ethanolic and Aqueous Extracts of Aesculus hippocastanum L. (Horse Chestnut) Bark against Bacteria Isolated from Urine of Patients Diagnosed Positive to Urinary Tract Infections. Front. Biosci. Scholar. 14, 10-11.

Lagacé, L., Pitre, M., Jacques, M., Roy, D., 2004. Identification of the bacterialcommunity of maple sap by using amplified ribosomal DNA (rDNA) restriction analysis and rDNA sequencing. Appl. Environ. Microbiol. 70, 2052–2060.

Lee, S., Mir, R.A., Park, S.H., Kim, D., Kim, H.-Y., Boughton, R.K., Jeong, K.C., 2020. Prevalence of extended-spectrum-lactamases in the local farm environment and livestock: Challenges to mitigate antimicrobial resistance. Crit. Rev. Microbiol. 46, 1-14.

Lelise, A., Belaynesh, G., Mulubrhan, M., Kedija, S., Endashaw, B., Abebe, B., 2014. Isolation and screening of antibacterial producing lactic acid bacteria from traditionally fermented drinks (ergo‖ and Tej‖) in Gondar town, Northwest Ethiopia, Global Research J. Epidmiol. Public Health, 3, 18-22.

Levy, S.B., Marshall, B., 2004. Antibacterial resistance worldwide: causes, challenges and responses. Nature Med., 10, S122–S129.

Manga, M.J.A., Podoprigora, I.V., Volina, E.G., Ermolaev, A.V., Smolyakova, L.A., 2021. Evaluation of changes induced in the probiotic Escherichia coli M17 following recurrent exposure to antimicrobials. J. Pharm. Res. Int. 33, 158-167.

Mbarga, M., Joseph, A., Podoprigora, I. V., Anyutoulou, K.L.D., Kezimana, P., Smolyakova, L.A., Hippolyte, T.M., Manar, R., Yashina, N.V., Smirnova, I.P., Irma, A.M.M., Das, M.S., 2022. Antimicrobial and Antibiotic-Resistance Reversal Activity of Some Medicinal Plants from Cameroon against Selected Resistant and Non-Resistant Uropathogenic Bacteria. Biosci. 14, 4- 25

Mir, F., Zaidi, A.K.M., 2009. Hospital infections by antimicrobial-resistant organisms in developing countries. In Sosa A, Byarugaba DK, Amabile-Cuevas C, Hsueh PR, Kariuki S and Okeke I (eds), Antimicrobial Resistance in Developing Countries. New York: Springer, pp. 347–362.

Monteagudo-Mera, A., Rodríguez-Aparicio, L., Rúa, J., Martínez-Blanco, H., Navasa, N., García-Armesto, M.R., Ferrero, M.Á., 2012. In vitro evaluation of physiological probiotic properties of different lactic acid bacteria strains of dairy and human origin. J. Funct. Foods 4, 531-541.

Motse, D.F.K., Ngaba, G.P., Foko, L.P.K., Ebongue, C.O., Adiogo, D.D., 2019. Etiologic profile and sensitivity pattern of germs responsible for urinary tract infection among under-five children in Douala, Cameroon: a HospitalBased Study. Avicenna J. Clin. Microbiol. 6, 49-56.

Mund, M.D., Khan, U.H., Tahir, U., Mustafa, B.-E., Fayyaz, A., 2017. Antimicrobial drug residues in poultry products and implications on public health: A review. Int. J. Food Prop. 20, 1433-1446.

Nagaraj, B., Kwang-Hyun, B., 2022. Combination Strategies of Different Antimicrobials An Efficient and Alternative Tool for Pathogen Inactivation Department of Biotechnology, Yeungnam University, Gyeongsan 38451

Noori, T.E., Alwan, M.J., 2016. Isolation and Identification of Zoonotic Bacteria from Poultry Meat. Int. J. Adv. Res. Biol. Sci. 3, 57-66.

Oluwatobi, F.B., Stephen, A.B., Fabulous, F.T.A., 2021. Isolation and Identification of Pathogenic Bacteria Associated with Raw Meat from Different Locations in Ado-Ekiti. Int. J. Res. Sci. Innov. Appl. Sci. 4, 24-54

Perez, R.H., Zendo, T., Sonomoto, K., 2014. Novel bacteriocins from lactic acid bacteria (LAB): various structures and applications. Microb. Cell Factor 13, 1-3.

Public Health England (PHE), 2009. Guidelines for Assessing the Microbiological Safety of Ready-to-Eat Foods Placed on the Market. Available online at: www.gov.uk/government/publications/ready-to-eat-foods-microbiological-safety-assessment-guidelines

Rabiul, I., Nur H., Khasrul, A., Ekhlas, U., Mehadi, H. R., Abu Sayeed, I., Firoz, A., 2020. Antibacterial Activity of Lactic Acid Bacteria and Extraction of Bacteriocin Protein. Adv. biosci. biotechnol. 11, 49-59.

Reda, F.M., El-Zawahry, Y.A., Omar, A.R., 2017. Synergistic effect of combined antibiotic and methanol extract of Eucalyptus camaldulensis leaf against Staphylococcus aureus and Pseudomonas aeruginosa. Int. J. Appl. Sci. Biotechnol. 5, 486-497.

Shahabe, A.S., Nabeeh Abdullah A., Irfan A., Suraj A., Shaik M.;, Mukhatar A. J., Nazima N., 2021. Synergistic antibacterial activity of herbal extracts with antibiotics on bacteria responsible for periodontitis. J. Infect. Dev. Ctries. 15, 1685-1693.

Shokryazdan, P., Sieo, C.C., Kalavathy, R., Liang, J.B., Alitheen, N.B., Faseleh Jahromi, M., Ho, Y.W., 2014. Probiotic potential of Lacto*Bacillus* strains with antimicrobial activity against some human pathogenic strains. BioMed Res. Inter. 2014.

Stenfors Arnesen, L. P., Fagerlund, A., Granum, P. E., 2008. From soil to gut: *Bacillus* cereus and its food poisoning toxins. FEMS Microbiol. 32, 579-606.

Usman P., Riftyan, E., Ayu, D.F., Jonnaidi, N.N., Wahyuni, M.S., Feruni, J.A., Abdel-Wahhab, M.A., 2021. Antibacterial efficacy of lactic acid bacteria and bacteriocin isolated from Dadih's against Staphylococcus aureus. Food Sci. Technol. 42, e27121.

Vishnuraj, M., Kandeepan, G., Rao, K., Chand, S., Kumbhar, V., 2016. Occurrence, public health hazards and detection methods ofantibiotic residues in foods of animal origin: A comprehensive review. Cogent. Food Agric. 2, 1235458.

Wassenaar, T.M., 2005. Use of antimicrobial agents in veterinary medicine and implications for human health. Crit. Rev. Microbiol. 31, 155–169.

WHO (World Health Organization), 2019. New Report Calls for Urgent Action to Avert Antimicrobial Resistance Crisis. Available at: https://www.who.int/news/item/29-04-2019-new-report-c alls-for-urgent-action-to-avert-antimicrobial-resistance-crisis.