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ANTAGONISTIC EFFECTS OF LACTIC ACID BACTERIA ON MULTI-DRUG RESISTANT PATHOGENS FROM CAT FAECES

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ABSTRACT

Cats being domestic animals are kept as companions of humans but harbour various microorganisms which could become pathogenic to the animal as well as the human companion by zoonotic transfer. An alternative to antibiotics as infectious agents is probiotics which are usually obtained from the same host in which it is intended for use and is currently being researched to check the continuous spread of multi-drug resistant bacteria. This study was conducted to isolate, identify and characterize lactic acid bacteria (LAB) from the faeces of cats and evaluate the antimicrobial effects of such isolated LAB on multidrug-resistant pathogens from the same animals. Faecal samples were randomly collected aseptically from apparently ten healthy cats into sterile universal bottles. Multidrug-resistant bacteria and LAB were isolated using appropriate agar media and identified by partial sequencing of the 16SrRNA gene. Antibiotic susceptibility pattern of LAB and bacteria isolates were determined by the agar diffusion method. The antibacterial activity of the LAB was determined against the test pathogens using the agar overlay and agar diffusion methods. Thirty species of LAB were isolated and identified as: *Lactobacillus plantarum* (14), *Lactobacillus plajomi* (1), *Weissella paramesenteroides* (4), *Lactobacillus paraplantum* (1), *Lactobacillus fermentum* (2), *Weissella confusa* (2), *Weissella cibaria* (2), *Enterococcus faecalis* (1), *Enterococcus durans* (2) and *Enterococcus hirae* (1). Bacteria pathogens were identified as *Bacillus cereus* C10, *Bacillus vietnamensis* I8, *Aerococcus viridans* I7, *Shigella flexneri* J4 and *Escherichia marmotae* B24. The LAB and bacteria isolates showed varying susceptibility pattern to antibiotics. Viable LAB cells and cell free supernatant (CSF) inhibited the growth of the test organisms. The viable LAB cells had better antibacterial activity than the CSF with the highest zone of inhibition of 35±0.5 mm against *Escherichia marmotae* B24 by *Weissella confusa* LC1. This study revealed that LAB from cat faeces possesses significant antimicrobial activity against multidrug-resistant pathogens from the same environment.

KEYWORDS: **Cat faeces; Lactic acid bacteria; Multidrug-resistant pathogens; Antibacterial activity.**

INTRODUCTION

Cats are domestic animals kept in homes as pets. This is an age long practice of man in the primitive times and in the present modern society. In some developing nations including Nigeria, cats are kept as companion animals and also for their ability to hunt down rodents that are destructive pests. However, cats are known to harbour various microorganisms including viruses, bacteria, parasites, and fungi which could become pathogenic to the animal as well as the human companion by zoonotic transfer [1]. Zoonosis, as jointly defined by World Health Organization (WHO) and Food and

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Agriculture Organization (FAO) expert committee on zoonosis, are "those diseases or infections that are naturally transmitted between vertebrate animals and man" [2]. Infectious agents from animal reservoir can be transferred to human through several ways such as animal bites, direct contact with animals, arthropod vectors like ticks, contacts with fur, hides or skin and indirect ingestion of contaminated materials such as: water, food, milk and meat [1]. The use of antibiotics for treatment of infectious diseases eliminates both the beneficial and the disease-causing bacteria leading to an imbalance in the gut microbiota, a situation referred to as "dysbiosis" known to result in a number of conditions such as irritable bowel syndrome and yeast infections. Indiscriminate use of antibiotics has led to the development of resistance by pathogens which were once susceptible to the antibiotics. The incidence of antimicrobial resistance (AMR) by microorganisms to available antibiotics/antimicrobial agents is on the rise and is one of the biggest threats to global health as many antibiotics are now less effective to treat infectious diseases. Antibiotic resistance leads to higher medical costs, prolonged hospital stays, and increased mortality. The burden of antimicrobial resistance is highest in developing countries in Asia and Africa [3]. This calls for caution in the use of available antibiotics and the search for effective alternatives. The use of probiotics as prophylaxis and treatment would be a better substitute to the use of antibiotics in treating zoonotic infections contracted either by direct or indirect contact with cats.

Probiotics are live microorganisms known to confer health benefits on the host when consumed in adequate amounts as part of food [4]. Live bacteria used as probiotics are expected to be separated from the same species as the intended host, have clear and favorable effect on the host, have the capacity to survive the conditions of the host's gut and not cause any infection/disease to the host. Three major bacteria species that have been extensively studied and used as probiotics in humans and animals are the Lactobacilli, bifidobacteria and Saccharomyces [5]. The importance of probiotics may be described as inexhaustible as they function widely by protecting against various disease-causing agents such as fungi, yeast and bacteria. They also aid digestion, improve immune function and balance hormonal level [6, 7]. Probiotics have ability to exert antimicrobial activity by producing substances that can inhibit the growth and multiplication of existing and invading organisms. These inhibitory substances include acids (acetic, citric, hippuric and lactic acids), diacetyl, hydrogen peroxide and bacteriocin all of which are known to possess antimicrobial properties [7]. Apart from producing bactericidal substances, they also compete with existing and invading pathogens and toxins for adherence to the intestinal epithelium. Probiotics maintain intestinal epithelial homeostasis by promoting epithelial cell survival, enhancing barrier function as well as stimulating protective responses from intestinal epithelial cells [8]. With these properties of probiotics, they could serve as effective substitutes for antibiotics in the treatment of infections.

Probiotics (lactic acid bacteria) have long been used in feed fermentation and as preservatives in food where they act as antimicrobials [9, 10]. Probiotics are now being explored as alternative therapeutic agents and are known to have better activity when isolated from the same host where they are intended to be used. Since cats are known to be involved in zoonotic transfer to humans, it is imperative to source for therapeutic agents from the animals which can be used to treat infection/diseases in the same animals as well as in the human companion. The aim of this study was to isolate and characterize lactic acid bacteria (LAB) with potential as probiotics from cat's faeces and evaluate such isolated LAB for their antagonistic effects against multi-drug resistant pathogens from same habitat.

MATERIALS AND METHODS

Collection of samples and isolation of bacteria

A random house-to-house and market visit was done in Ibadan, Oyo state between June and July, 2016 to collect faecal samples aseptically from apparently ten healthy cats into sterile universal bottles. The samples were taken to the laboratory within one hour of collection for analysis. One gram (1 g) of the cat faeces was added to 9 mL of sterile peptone water and homogenized with a vortex mixer. Tenfold dilutions were carried out and 1 mL each from 10^{-6} dilutions of homogenized mixture of each sample was inoculated into: (i) Man, Rogosa and Sharpe (MRS) agar (Oxoid, UK) by pour plate method and incubated at 37 °C for 48 h under microaerophilic condition using CampyGen™ (Oxoid, UK) to isolate lactic acid bacteria (LAB), and (ii) nutrient agar and MacConkey agar by pour plate method and incubated at 37 °C under aerobic condition for the isolation of potential pathogens.

For the isolation of lactic acid bacteria, colonies were picked according to difference in sizes and shapes on MRS agar plates. All strains under examination were tested for Gram staining reaction and catalase

production. Only Gram positive and catalase-negative isolates were selected for further work. Pure cultures presumed to be lactic acid bacteria were kept for long term storage in Eppendorf tubes containing MRS broth with 50 % glycerol at -20 ^{o}C. The tentative determination of the pathogens was done by growing on differential media such as mannitol salt agar (MSA), MacConkey agar (MCA) and Eosin Methylene Blue Agar (EMB); and incubated aerobically at 37 ^{o}C for 24 h.

Viable count of lactic acid bacteria and isolated bacteria pathogens

After incubation under microaerophilic condition at 37 ^{o}C for 48 h, colonies were counted based on differences in their morphology on MRS agar plates to determine the colony forming unit of lactic acid bacteria per gram of the cat faeces. The pathogens were grown on nutrient agar plates and colonies counted to determine the colony forming unit per gram (cfu/g) of faeces.

Identification of isolates by sequencing of 16SrRNA gene

The DNA of the isolates were extracted using *AccuPrep*® Genomic DNA Extraction Kit (Bioneer Corporation, South Korea) following the manufacturer's instruction. Genetic identification of LAB isolates and some pathogens were performed by Polymerase Chain Reaction (PCR) targeted to the 16SrRNA gene using forward primer: BSF-8 (5'AGAGTTTGATCCTGGCTCAG-3') and reverse primer: BSR-534 (5'-ATTACCGCGGCTGCTGGC-3'). The PCR condition described by [11] used was. The gel was stained for 10 min in ethidium bromide, de-stained for 20 min in distilled water and viewed using a UV transilluminator (UVP GelMax™ Imager). The PCR products were purified and sequenced for identification using the Sanger single read sequencing at GATC biotech Germany. The sequences were compared with NCBI data base by running a mega blast of 16S ribosomal RNA sequences (bacteria and archaea) of highly similar sequences with the default algorithm parameters.

Determination of antibiotic susceptibility of lactic acid bacteria isolated from cat faeces

The susceptibility of the isolated lactic acid bacteria (LAB) against some selected antibiotics was determined by the disk diffusion method [12]. Eighteen millilitres (18 mL) of MRS agar were poured into Petri dish and allowed to set. The LAB culture was spread on to the solidified agar by means of sterile swab stick. The antibiotic disk containing ceftazidine-30 µg, cefuroxine-30 µg, gentamicin-10 µg, ceftriaxone-30 µg, erythromycin-5 µg, cloxacillin-5 µg, ofloxacin-5 µg, Augmentin®-30 µg was placed firmly on the surface of the solidified MRS agar with the aid of a sterile forceps and incubated at 37 ^{o}C for 24 h under microaerophilic condition. The susceptibility of the test organisms (LAB) to the selected antibiotics was shown by clear zones of inhibition around the antibiotic disks.

Determination of the antibiotic susceptibility of the pathogen

The pathogens isolated from cat faeces were screened against eight antibiotics using the disc diffusion methods [13]. Twenty millilitres (20 mL) of Mueller Hinton agar were poured into a sterile Petri dish and allowed to set. The isolates were adjusted to 0.5 McFarland standards and inoculated on the plates using a sterile swab stick. The antibiotic discs (Abtek Biologicals Ltd., UK) containing ceftazidime (30 µg), cefuroxime (30 µg), gentamicin (10 µg), cefixime (5 µg), nitrofurantoin (300 µg), ciprofloxacin (5 µg), ofloxacin (5 µg) and Augmentin® (30 µg) were placed firmly on the surface of the agar using sterile forceps and incubated aerobically for 24 h. The susceptibility of the test organisms to the antibiotics was evident by clear zones of inhibition around the antibiotic discs. The results were interpreted using the clinical laboratory standard institute guidelines (CLSI) 2016 [13].

Determination of the antimicrobial activity of viable cells of lactic acid bacteria and cell-free supernatant

The antimicrobial activities of viable cells of LAB isolates and cell-free supernatant against pathogens isolated from the same habitat were investigated in *Bacillus cereus* C10*, Bacillus vietnamensis* I8, *Aerococcus viridans* I7*, Shigella flexneri* J4 and *Escherichia marmotae* B24 using the agar overlay and agar diffusion methods, respectively as described [11]. For the agar diffusion method, 20 mL of Mueller Hinton agar was poured into a sterile dish and allowed to set. The pathogen was spread over the plate using a sterile swab, then a sterile cork-borer was used to bore holes of 8 mm and the holes were filled with supernatants of the LAB isolates and incubated at 37^{o}C for 24 h.

The supernatant of the LAB was obtained by spinning overnight broth culture of LAB at 10,000 rpm for 10 min. Clear zones of inhibition indicated susceptibility of the pathogens to the LAB isolates.

Statistical analysis

Antimicrobial assay of isolated LAB against test pathogens were done in two different replicates and the Standard Error of the Mean (SEM) of diameter of zones of inhibition was calculated. Data were analyzed by one way ANOVA at $\alpha_{0.05}$.

RESULTS

Presumptive lactic acid bacteria were isolated from ten faecal samples collected from different cats for the determination of their antagonistic activities against multidrug-resistant pathogens isolated from the same environment. The isolates were presumptively identified as lactic acid bacteria by morphological characteristics on MRS agar, Gram stain reaction and catalase test. Isolates with small circular morphology, cream to off white colours, raised, smooth with entire margin colonies, which were also positive for Gram reaction and were catalase negative were selected for further investigation. The colony forming unit per gram of the LAB isolates recorded ranged from 1.25×10^4 to 2.6×10^5. Thirty (30) LAB isolates were identified using molecular method by partial sequencing of the 16SrRNA gene. *Lactobacillus plantarum* had the highest percentage (46.7 %) occurrence followed by *Weisella paramesenteroides* (13.3 %). *Lactobacillus fermentum*, *Weissella confusa*, *Weissella cibaria* and *Enterococcus durans* had 6.7% occurrence each while, *Lactobacillus plajomi*, *Lactobacillus paraplantum*, *Enterococcus faecalis* and *Enterococcus hirae* had the least percentage (3.3 % each) occurrence (Figure 1). Table 1 shows the description and percentage accuracy of the isolates' sequences as retrieved from the NCBI database using BLAST.

Percentage distribution of lactic acid bacteria

Augmentin had 100% inhibitory activity on the LAB followed by erythromycin with 98% inhibitory activity. The LAB isolates showed 83.3 % and 66.7 % resistance to cloxacillin and ofloxacin, respectively (Table 2). *Lactobacillus plantarum* LD3 was resistance to all antibiotics except erythromycin and Augmentin®. *Enterococcus hirae* LC8 was susceptible to all the antibiotics except gentamicin while, *Enterococcus durans* LH7 and *Weissella confusa* LG8 were susceptible to all the antibiotics except cloxacillin (Table 2).

The colony forming unit per gram (cfu/g) of the presumed pathogens carried out on the cat's faecal sample showed varying results from 1.5×10^3 to 2.0×10^5. About 76 isolates were initially cultured and 10 were selected for further study based on the antibiogram results of the isolates. The susceptibility of isolated pathogens to different antibiotics was determined using disc diffusion method and the results were interpreted using CLSI 2016 guidelines. Pathogens that were resistant to more than three classes of antibiotics (Table 3) were further identified by partial sequencing of their 16SrRNA genes as *Bacillus cereus* C10, *Bacillus vietnamensis* I8, *Aerococcus viridans* I7, *Shigella flexneri* J4 and *Escherichia marmotae* B24.

The viable LAB isolates and the cell free supernatants (CFS) were tested for their antimicrobial activity against the MDR isolated pathogens. The diameter of the zones of inhibition as seen in Tables 4 and 5 showed that the viable LAB cells and CFS have varying antibacterial activity against the MDR pathogens. The viable LAB cells had better antibacterial activity (radius of zone of inhibition was 6 $\pm$ 0.0 to 35 $\pm$ 0.5 mm) than the cell free supernatants (diameter of zone of inhibition = 6 $\pm$ 0.0 to 21 $\pm$ 0.0 mm) (Tables 4 and 5). The highest antibacterial activity was demonstrated by *Weissella confusa* LC1 followed by *Lactobacillus plantarum* LB1 against *Escherichia marmotae* B24. *Escherichia marmotae* B24 was the most susceptible pathogen to all the LAB isolates while *Shigella flexneri* J4 was the least susceptible (Table 4).

DISCUSSION

Cats being domestic animals are companions of humans and are known to harbour various microorganisms including viruses, bacteria, parasites, and fungi which could become pathogenic to the animal as well as the human companion by zoonotic transfer [1]. Infectious agents from animal reservoir can be transferred to human through many different ways such as animal bites, direct contact with animals, arthropod vectors like ticks, contacts with fur, hides or skin and saliva as in the case of cats as well as indirect ingestion of contaminated materials such as water, food, milk and meat [1]. Zoonotic infections like any other are usually treated with antibiotics which have been reported over time to eliminate both the beneficial and pathogenic bacteria thus altering the gut microbiota which can result in a number of conditions such as irritable bowel syndrome and yeast infections. The continuous and indiscriminate use of antibiotics in

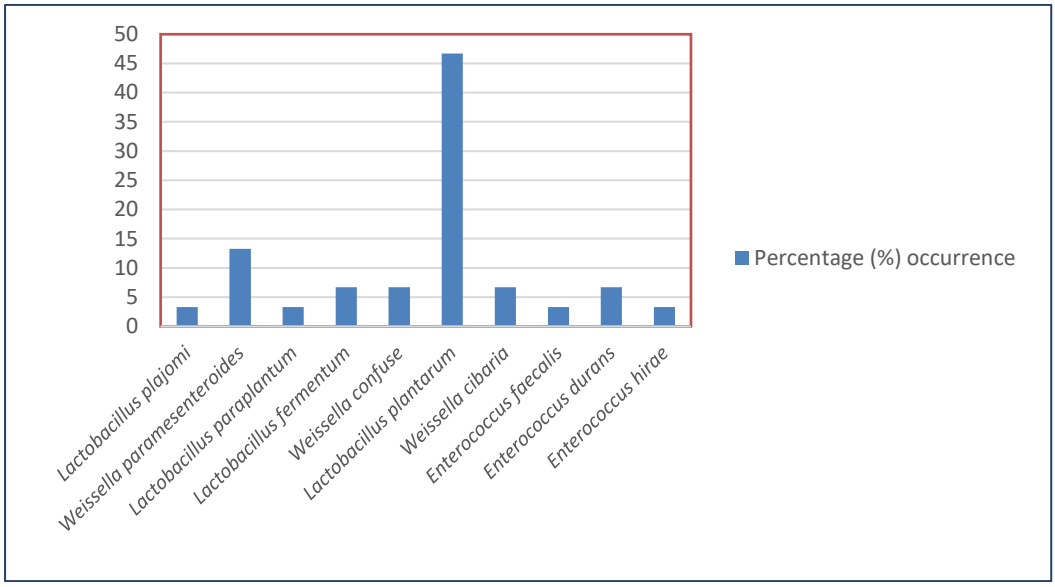

Figure 1: Percentage distribution of lactic acid bacteria (LAB) isolated and characterized from cat faeces showing *Lactobacillus plantarum* with the highest percentage (46.7 %) occurrence.

Table 1: Description of lactic acid bacteria (LAB) isolates

Isolate	Description	% Accuracy
LB1	*Lactobacillus plantarum*	98
LB5	*L. plantarum*	99
LB10	*L. plantarum*	99
LB11	*L. plantarum*	99
LB14	*L. plantarum*	99
LB15	*L. plantarum*	99
LC1	*Weissella confusa*	99
LC2	*Lactobacillus fermentum*	99
LC8	*Enterococcus hirae*	100
LC10	*Enterococcus durans*	97
LD3	*L. plantarum*	99
LD6	*L. plantarum*	99
LD8	*Enterococcus faecalis*	99
LD9	*Lactobacillus paraplantum*	98
LD10	*L. plantarum*	98
LD12	*L. plantarum*	99
LE11	*Weissella cibaria*	99
LF3	*L. plantarum*	99
LG8	*Weissella confusa*	99
LH3	*Weissella paramesenteroides*	99
LH6	*Weissella paramesenteroides*	99
LH7	*Enterococcus durans*	99
LH8	*Weissella paramesenteroides*	97
LH11	*L. plantarum*	99
LH12	*L. plantarum*	98
LH14	*Weissella paramesenteroides*	90
LH15	*Weissella cibaria*	97
LI2	*Lactobacillus fermentum*	99
LI4	*L. plantarum*	100
LJ8	*Lactobacillus plajomi*	91

Table 2: Antibiotics susceptibility pattern of isolated LAB strains. Diameter (mm) of zone of inhibition ± SEM.

Lactic acid bacteria	CAZ	CRX	GEN	CTR	ERY	CXC	OFL	AUG
Lactobacillus plantarum LB1	15 ± 0.5	20 ± 0.0	13 ± 0.5	25 ± 0.5	16 ± 0.0	R	R	20 ± 0.0
L. plantarum LB5	15 ± 1.0	20 ± 0.5	8 ± 0.0	R	20 ± 0.5	R	R	20 ± 0.5
L. plantarum LB10	10 ± 0.0	15 ± 0.0	14 ± 0.5	20 ± 0.5	20 ± 0.0	R	R	28 ± 0.0
L. plantarum LB11	15 ± 1.0	R	14 ± 0.5	18 ± 0.5	20 ± 0.5	R	R	26 ± 0.5
L. plantarum LB14	10 ± 0.5	14 ± 1.0	10 ± 0.0	14 ± 0.0	19 ± 1.0	R	R	20 ± 0.5
L. plantarum LB15	R	R	7 ± 0.0	R	16 ± 1.0	R	R	20 ± 1.0
Weissella confusa LC1	R	R	R	10 ± 0.5	20 ± 0.5	R	11 ± 0.5	26 ± 0.5
L. fermentum LC2	R	R	14 ± 1.0	10 ± 0.5	22 ± 0.5	R	9 ± 0.0	25 ± 0.5
Enterococcus hirae LC8	7 ± 0.0	17 ± 0.0	R	17 ± 0.0	20 ± 1.0	15 ± 0.0	15 ± 0.5	28 ± 0.0
E. durans LC10	R	R	R	12 ± 0.5	18 ± 0.0	R	10 ± 0.5	20 ± 1.0
L. plantarum LD3	R	R	R	R	16 ± 0.0	R	R	20 ± 0.5
L. plantarum LD6	R	R	10 ± 0.5	R	20 ± 1.0	R	R	25 ± 0.0
E. faecalis LD8	R	15 ± 1.0	R	10 ± 0.5	R	R	10 ± 0.5	25 ± 0.5
L. paraplantum LD9	R	R	10 ± 0.5	19 ± 1.0	19 ± 0.0	R	R	20 ± 0.5
L. plantarum LD10	R	R	14 ± 0.5	R	18 ± 0.0	R	9 ± 0.5	20 ± 0.0
L. plantarum LD12	17 ± 1.0	22 ± 0.0	20 ± 0.5	30 ± 0.5	25 ± 0.5	9 ± 0.5	R	30 ± 0.5
W. cibaria LE11	10 ± 0.5	R	11 ± 1.0	14 ± 1.0	15 ± 0.0	R	R	20 ± 0.0
L. plantarum LF3	R	10 ± 0.0	R	14 ± 0.5	20 ± 1.0	9 ± 0.0	15	25 ± 1.0
W. confusa LG8	16 ± 1.0	20 ± 0.5	10 ± 1.0	21 ± 0.0	15 ± 1.0	R	10 ± 1.0	20 ± 0.0
W. paramesenteroides LH3	R	16 ± 0.5	R	15 ± 1.0	20 ± 0.5	10 ± 0.5	10 ± 1.0	25 ± 1.0
W. paramesenteroides LH6	10 ± 0.5	10 ± 1.0	**7** ± 0.0	14 ± 1.0	20 ± 0.5	R	R	20 ± 0.5
E. durans LH7	9 ± 1.0	15 ± 1.0	9 ± 0.0	15 ± 0.5	20 ± 0.0	R	10 ± 1.0	20 ± 0.0
L. plantarum LH11	R	R	10 ± 0.5	18 ± 0.0	19 ± 0.5	R	R	21 ± 1.0
L. plantarum LH12	R	R	**7** ± 0.0	20 ± 1.0	18 ± 1.0	R	R	22 ± 1.0
W. paramesenteroides LH14	15	16 ± 0.5	R	15 ± 0.0	19 ± 0.5	14 ± 1.0	R	20 ± 0.5
W. cibaria LH15	15 ± 1.0	15 ± 1.0	**7** ± 0.5	10 ± 0.5	15 ± 0.0	R	R	18 ± 1.0
L. fermentum LI2	R	R	11 ± 0.5	9 ± 1.0	20 ± 1.0	R	R	24 ± 0.5
L. plantarum LI4	13 ± 0.5	19 ± 0.5	R	20 ± 0.5	20 ± 0.5	R	R	26 ± 0.5
L. plajomi LJ8	9 ± 1.0	9 ± 0.5	9 ± 0.5	15 ± 0.5	20 ± 0.0	R	R	26 ± 1.0

Key: CAZ- Ceftazidine 30 µg, CRX- Cefuroxine 30 µg, GEN- Gentamycin 10 µg, CTR- Ceftriaxone 30 µg, ERY: Erythromycin 5 µg, CXC- Cloxacillin 5µg, OFL- Ofloxacin 5 µg, AUG- Augmentin® 30 µg, R- Resistance

Table 3: Antibiotics susceptibility pattern of pathogens isolated from cat faeces

Isolate	CAZ	CRX	GEN	CTR	ERY	CXC	OFL	AUG
Bacillus cereus C10	6, R	6, R	11, R	6, R	9, R	6, R	20, S	30, S
B. vietnamensis I8	6, R	6, R	10, R	6, R	9, R	6, R	15, I	14, I
Aerococccus viridans I7	12, R	20, S	20, S	20, I	20, S	26, S	28, S	30, S
	CAZ	**CRX**	**GEN**	**CXM**	**OFL**	**AUG**	**NIT**	**CPR**
Shigella flexneri J4	20, I	22, S	20, S	14, R	6, R	20, S	20, S	6, R
Escherichia marmotae B24	6, R	6, R	14, I	6, R	26, S	6, R	6, R	27, S

Key: CAZ= ceftazidime (30 µg), CRX= cefuroxime (30 µg), GEN= gentamicin (10 µg), CTR= Ceftriaxone (30 µg), ERY= Erythromycin (5 µg), CXC= Cloxacillin (5µg), OFL= ofloxacin (5 µg), AUG= Augmentin® (30 µg), CXM= cefixime (5 µg), NIT= nitrofurantoin (300 µg), CPR= ciprofloxacin (5 µg), S=Sensitive; R= Resistant; I= Intermediate [CLSI, 2016].

Table 4: Inhibition of test pathogens by viable cells of LAB from cat faeces. Radius (mm) of zone of inhibition ± SEM

Isolated lactic acid bacteria	***Escherichia marmotae* B24**	***Bacillus cereus* C10**	***Shigella flexneri* J4**
Lactobacillus plantarum LB1	34 ± 0.0	20 ± 1.0	10 ± 0.0
L. plantarum LB5	25 ± 0.0	17 ± 0.0	16 ± 0.0
L. plantarum LB10	24 ± 0.5	17 ± 0.5	15 ± 0.0
L. plantarum LB11	30 ± 1.0	15 ± 1.0	9 ± 1.0
L. plantarum LB14	30 ± 1.0	10± 0.0	15 ± 0.0
L. plantarum LB15	30 ± 0.0	12 ± 0.0	10 ± 1.0
Weissella confusa LC1	35 ± 0.5	15 ± 1.0	9 ± 0.0
L. fermentum LC2	23 ± 0.0	14 ± 0.0	12 ± 0.5
Enterococcus hirae LC8	20 ± 1.0	12 ± 0.0	10 ± 1.0
E. durans LC10	25 ± 1.0	12 ± 1.0	9 ± 0.0
L. plantarum LD3	18 ± 0.0	15 ± 0.0	16 ± 1.0
L. plantarum LD6	23 ± 0.0	15 ± 0.0	15 ± 0.5
E. faecalis LD8	25 ± 0.5	10 ± 0.5	15 ± 1.0
L. paraplantum LD9	25 ± 0.5	17 ± 0.0	20 ± 1.0
L. plantarum LD10	30 ± 0.0	17 ± 0.0	22 ± 0.0
L. plantarum LD12	27 ± 1.0	13 ± 1.0	12 ± 0.0
W. cibaria LE11	30 ± 0.0	10 ± 1.0	10 ± 1.0
L. plantarum LF3	26 ± 1.0	10 ± 0.0	10 ± 0.0
W. confusa LG8	32 ± 0.0	8 ± 0.0	9 ± 1.0
W. paramesenteroides LH3	20 ± 0.0	10 ± 0.0	10 ± 0.0
W. paramesenteroides LH6	15 ± 0.0	10 ± 0.0	8 ± 0.0
E. durans LH7	15 ± 0.0	10 ± 0.0	10 ± 0.0
W. paramesenteroides LH8	22 ± 1.0	15 ± 0.5	7 ± 0.0
L. plantarum LH11	30 ± 0.0	12 ± 0.0	15 ± 0.0
L. plantarum LH12	32 ± 0.0	15 ± 0.5	13 ± 1.0
W. paramesenteroides LH14	30 ± 1.0	10 ± 0.0	9 ± 0.5
W. cibaria LH15	NZI	11 ± 0.0	12 ± 0.0
L. fermentum LI2	22 ± 0.0	17 ± 0.0	10 ± 0.0
L. plantarum LI4	30 ± 0.5	12 ± 0.5	15 ± 1.0
L. plajomi LJ8	28 ± 0.0	10 ± 1.0	14 ± 0.0
Ceftazidime (30 µg)	6 ± 0.0	6 ± 0.0	20 ± 0.0

Key: NZI – No zone of inhibition. *The diameter of zone of inhibition was significantly higher with $p < 0.05$.

Table 5: Inhibition of test pathogens by cell free supernatants of LAB strains. Diameter (mm) of zone of inhibition ± SEM

Isolated lactic acid bacteria	**Escherichia marmotae* **B24**	*Shigella flexneri* **J4**	**Bacillus vietnamensis* **I8**	**Aerococcus viridans* **I7**
Lactobacillus plantarum LB1	11 ± 0.0	11 ± 1.0	14 ± 0.5	14 ± 1.0
L. plantarum LB5	14 ± 1.0	12 ± 1.0	20 ± 0.5	15 ± 0.0
L. plantarum LB10	17 ± 0.5	12 ± 0.0	14 ± 0.0	15 ± 0.0
L. plantarum LB11	14 ± 0.0	12 ± 0.0	13 ± 0.0	12 ± 1.0
L. plantarum LB14	NZI	10 ± 0.5	16 ± 0.5	15 ± 0.5
L. plantarum LB15	11 ± 0.0	16 ± 0.5	15 ± 0.0	13 ± 1.0
Weissella confusa LC1	12 ± 0.0	09 ± 1.0	14 ± 0.0	14 ± 0.0
L. fermentum LC2	NZI	NZI	09 ± 1.0	10 ± 1.0
Enterococcus hirae LC8	NZI	NZI	12 ± 0.5	NZI
E. durans LC10	12 ± 0.5	09 ± 1.0	12 ± 1.0	10 ± 0.0
L. plantarum LD3	12 ± 0.0	13 ± 1.0	21 ± 0.0	12 ± 1.0
L. plantarum LD6	14 ± 0.5	14 ± 1.0	20 ± 1.0	NZI
E. faecalis LD8	13 ± 0.0	09 ± 0.0	10 ± 0.0	NZI
L. paraplantum LD9	11 ± 0.5	11 ± 0.5	15 ± 1.0	NZI
L. plantarum LD10	15 ± 0.5	14 ± 0.5	13 ± 1.0	NZI
L. plantarum LD12	14 ± 1.0	13 ± 1.0	10 ± 1.0	NZI
W. cibaria LE11	13 ± 1.0	11 ± 1.0	13 ± 0.5	NZI
L. plantarum LF3	NZI	NZI	13 ± 0.0	NZI
W. confusa LG8	13 ± 0.5	10 ± 0.5	15 ± 1.0	NZI
W. paramesenteroides LH3	12 ± 0.5	NZI	20 ± 0.5	19 ± 0.0
W. paramesenteroides LH6	NZI	NZI	20 ± 0.0	15 ± 0.5
E. durans LH7	NZI	11 ± 0.5	14 ± 1.0	15 ± 1.0
W. paramesenteroides LH8	NZI	10 ± 0.5	15 ± 0.0	15 ± 0.0
L. plantarum LH11	15 ± 0.0	15 ± 1.0	10 ± 0.5	NZI
L. plantarum LH12	13 ± 1.0	17 ± 1.0	14 ± 0.0	12 ± 0.5
W. paramesenteroides LH14	NZI	09 ± 0.5	16 ± 1.0	17 ± 0.5
W. cibaria LH15	NZI	NZI	16 ± 0.0	14 ± 1.0
L. fermentum LI2	11 ± 1.0	10± 1.0	14 ± 0.5	15 ± 0.5
L. plantarum LI4	09 ± 1.0	11 ± 1.0	09 ± 0.0	NZI
L. plajomi LJ8	11 ± 0.5	12 ± 1.0	15 ± 1.0	13 ± 1.0
Ceftazidime (30 µg)	6 ± 0.0	20 ± 0.0	6 ± 0.0	12 ± 0.0

Key: Diameter of cork borer = 8 mm; NZI – No zone of inhibition. *The diameter of zone of inhibition was significantly higher with $p < 0.05$.

man and animals have resulted in antimicrobial resistance, the incidence of which is on the rise and has constituted one of the biggest threats to global health as many antibiotics are now less effective to treat infectious diseases [3]. This calls for caution in the use of available antibiotics and the search for effective alternatives. The use of probiotics- live microorganisms known to confer health benefits on the host when consumed in adequate amounts- as prophylaxis and treatment would be a better substitute to the use of antibiotics in treating zoonotic infections contracted either by direct or indirect contact with cats as probiotics have been reported to demonstrate antimicrobial activity against pathogens from the same habitat. Probiotics mainly, lactic acid bacteria (LAB) have been used in food and feed fermentation processes long before it was recorded that the bacteria existed [9]. They are found in many nutrient rich environments and occur naturally in various food products such as dairy and meat products, and vegetables [10]. From several studies, lactic acid bacteria generally have been recognized as candidate probiotics for pets [14 - 16].

In this study, we investigated the antibacterial effect of lactic acid bacteria (LAB) isolated from the faeces of cat against multi-drug resistant pathogens from same habitat. Thirty species of lactic acid bacteria were isolated, identified and characterized. The three genera of lactic acid bacteria that were identified are Lactobacilli, Weissella and Enterococci. *Lactobacillus plantarum* had the highest percentage (46.7%) occurrence, followed by *Weisella paramesenteroides* (13.3%). *Lactobacillus plajomi*, *Lactobacillus paraplantum*, *Enterococcus faecalis* and *Enterococcus hirae* had the least (3.3% each) occurrence. *Lactobacillus fermentum*, *Weissella confusa*, *Weissella cibaria* and *Enterococcus durans* had 6.7% occurrence each (Figure 1). *Lactobacillus* species were most dominant lactic acid bacteria isolated in this study. This finding correlates with the report of Jia *et al*. [17] whose research was carried out on kittens. *Lactobacillus* was found in high numbers in the faeces of healthy adult cats according to Lubbs *et al.* [18]. Ritchie *et al*. [19] reported *Lactobacillus spp*. in 92% of pet cats using Lactobacillus-specific primers. *Enterococcus faecalis* have been isolated severally from cheese, fish, sausages, minced beef and pork [20, 21] and are used in animal, mainly poultry and pig nutrition. There are also pharmaceutical products that contain *Enterococcus spp*. as probiotics. Some researchers have reported the antimicrobial competence of *Weissella* spp. against uropathogens. *Weissella cibaria* in particular has been demonstrated to inhibit the formation of dental biofilm [22] and genitourinary pathogens [23].

Lactic acid bacteria are renowned for their ability to produce antimicrobial compound and other value-added products. The antimicrobials produced are known to have strong antagonistic activity against many Gram-positive and Gram-negative bacteria [24] and fungi [25]. All the lactic acid bacteria isolated in this study showed significant and varying antagonistic activity against the identified multi-drug resistant bacteria isolated from the same environment. The antagonistic effects are evident by the large zones of inhibition of growth of the test organism by the isolated lactic acid bacteria (Table 4). The whole viable cell had better activity than the cell free supernatant (Tables 4 &5). Resistance of LAB isolates from human and animals to antibiotics is an important characteristic of LAB if they must be used as probiotics. Our findings showed the LAB isolates had 83.3% and 66.7% resistance to cloxacillin and ofloxacin, respectively (Table 2). *Lactobacillus plantarum* LD3 was resistance to all antibiotics except erythromycin and Augmentin®. Antibiotics resistance of LAB isolates from human and animal have also been reported [26, 27].

The emergence and increasing spread of MDR bacteria among household pets including cats in recent times is a growing concern as there exist numerous connections between MDR pathogens from human and domestic pets' infections suggesting zoonoses resulting from direct and indirect contact [28]. The bacteria pathogens, *Bacillus cereus* C10*, Bacillus vietnamensis* I8, *Aerococcus viridans* I7*, Shigella flexneri* J4 and *Escherichia marmotae* B24 isolated from cats' faeces in this study are all potential pathogens in humans should they be transferred from cat to humans. Lactic acid bacteria, as shown in this study demonstrated antimicrobial activity against the multi-drug resistant pathogens in this study. The lactic acid bacteria and the pathogens were isolated from the same habitat. This suggests that lactic acid bacteria from cat gastrointestinal tract could serve as potential probiotics for cats without the fear of encouraging microbial antibiotics resistance that is transferable to humans. The findings in this study warrants further research to ascertain that the isolated and identified lactic acid bacteria fulfil other criteria required by the WHO and FAO for the selection of probiotic strains in animals.

CONCLUSION

The study investigated the antibacterial activities of lactic acid bacteria (LAB) isolated, identified and characterized from the faeces of cats against

pathogenic bacteria isolated from the same source. Thirty species of lactic acid bacteria were isolated belonging to three different genera of lactic acid bacteria which are Lactobacilli, Weissella and Enterococci. Multidrug-resistant pathogens isolated from the same source are *Bacillus cereus* C10*, Bacillus vietnamensis* I8, *Aerococcus viridans* I7*, Shigella flexneri* J4 and *Escherichia marmotae* B24. Antibacterial evaluation of the LAB revealed that whole viable LAB cells had better activity than the cell free supernatant. These findings suggest that lactic acid bacteria from cat gastrointestinal tract could serve as potential probiotics for cats without the fear of encouraging microbial antibiotics resistance that is transferable to humans.

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