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Research Article

Antibacterial Activities of Lactic Acid Bacteria on Potential Multidrug-resistant Pathogens Isolated from Rabbit

Aizebeoje C.I., *Lawal T.O. and Adeniyi B.A.
Department of Pharmaceutical Microbiology, University of Ibadan, Nigeria

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Abstract
Rabbits are raised as food animal in Nigeria; and the possibility of transfer of infection(s) to humans cannot be ruled out. The overuse and abuse of antibiotics in treating zoonotic infections has contributed to the rise in antimicrobial drug resistance, therefore, alternatives to antibiotics are needed. The study was carried out to determine the antibacterial activity of lactic acid bacteria (LAB) isolated from rabbit's faeces against multidrug-resistant (MDR) pathogens isolated from the same rabbit. Twelve (12) faecal samples and twelve (12) fur swabs samples were randomly collected aseptically from apparently healthy rabbits from Ajibode, Ibadan and Research farm, University of Ibadan, Oyo state, Nigeria. Lactic acid bacteria and multidrug-resistant pathogens were isolated using appropriate agar media and identified by partial sequencing of the 16S rRNA gene. Antibiotic susceptibility pattern of isolated bacteria and LAB were determined by the agar diffusion method. Antibacterial activity of the LAB against the test pathogens was determined using agar overlay and agar diffusion methods. The pathogens *Myroides gitamensis* and *Citrobacter rodentium* as well as twenty (20) species of LAB belonging to Lactobacillus genus were identified and characterized. *Lactobacillus plantarum* had the highest (60.71%) occurrence among the LAB. Viable cells and cell free supernatant (CFS) of isolated LAB inhibited the growth of the test organisms with the largest zone of inhibition (40 + 0.0 mm) produced by *Lactobacillus plantarum* against *Citrobacter rodentium*. This study showed that LAB from rabbit possess considerable antibacterial activity against multidrug-resistant bacteria from the same environment hence, can be suitable alternatives to antibiotics.

Key Words: Rabbits' faeces; Lactic acid bacteria; Multidrug-resistant gut pathogens; Cell-free supernatant; Antibacterial activities

INTRODUCTION

Rabbits are raised as food animal in Nigeria; and the possibility of transfer of infection (s) to humans cannot be ruled out should they be infected by pathogenic organisms. Rabbits could become susceptible to infecting microorganisms to which other animals as well as humans are susceptible. These pathogens include respiratory *Pasteurella multocida*, *Bordetella bronchiseptica*, *Moxarella catarrhalis* and *Escherichia coli* which cause diseases in rabbits (Deeb, 2000). Many people come in contact with animals in their daily lives when they are raised for food or kept as pets with the possibility of contracting infections from these interactions. The infections are usually treated with antibiotics many of which are lethal to the invading bacteria as well as the protecting (resident) bacteria. Many of the antibiotics are also known to disturb the natural balance of the gut thus reducing the population of the resident bacteria that keep the gastrointestinal immune defenses strong and resilient (Ubeda and Pamer, 2012). Although antibiotics are lifesaving, the drastic surge in their use have created some new problems such as weakened immune systems and digestive problems resulting from disturbances in the balance of the protective flora of the gastrointestinal and vaginal tracts (Ubeda and Pamer, 2012). The number of pathogens developing resistance to many of the available antibiotics due to the widespread misuse of antibiotics are on the rise hence, the need to source for effective alternative agents from natural sources. Probiotics which are live microorganisms (bacteria) have been reported to confer health benefits to the recipients when administered in adequate quantity (Reid *et al.*, 2014). These may constitute safe alternative to antibiotics as they have been observed to prevent invasion by pathogenic microorganisms both in humans and in rabbit by competing for nutrients and space, and as well produce antimicrobial substances while maintaining microbial balance (Vanderpool *et al.*, 2008; Yan and Polk, 2011).

Probiotics are also known to possess antagonistic activities against various Gram-positive and Gram-negative bacterial pathogens as well as reduce the risk of disorders from bacterial pathogens in the gut (van Zyl *et al.*, 2020). Lactic acid bacteria (LAB) and bifidobacteria are the most commonly used

**Author for Correspondence:* Tel: +2348066591756 E-mail: *lawaltemitope8@gmail.com*

probiotics (Linares *et al.*, 2017). Lactic acid bacteria are Gram-positive, fermentative and microaerophilic microorganisms, the metabolic activities of which result in production of high amounts of organic acids (Mora-Villalobos *et al.*, 2020).

Probiotics could effectively serve as biotherapeutic and safe alternatives to antibiotics for the prevention and treatment of infections in rabbits as well as humans. In this study, lactic acid bacteria from the faeces and fur swabs of rabbits were isolated, characterized, identified and tested for possible antibacterial activity in bacteria pathogens isolated from the same source.

MATERIALS AND METHODS

Collection of Samples: Twenty-four (24) samples comprising twelve (12) faecal samples and twelve (12) fur swab samples, were randomly collected aseptically from apparently healthy rabbits from Ajibode, Ibadan and University of Ibadan research farm in Ibadan, Oyo state, using sterile universal bottles and sterile swab sticks. Samples were collected between May and August, 2016. Within one hour of collection, all samples were taken to the laboratory for microbiological analysis.

Isolation procedures and phenotypic identification of lactic acid bacteria (LAB): One (1) gram of the rabbit faeces was added to 9 mL of de Man Rogosa Sharpe broth in tubes and incubated at 37ºC for 24 h in a microaerophilic environment. Appropriate serial dilution was carried out using sterile normal saline. One milliliter (1 mL) of 10^{-5} dilutions of overnight culture of homogenized faecal sample was added to sterile molten MRS agar (Oxoid, UK) and poured into sterile Petri dish. Inoculated agar in Petri dishes were left to set firmly before incubating at 37ºC for 48 h in a microaerophilic environment using CampyGen (Oxoid). One milliliter (1 mL) of 10^{-4} dilutions of overnight culture of swab samples were transferred on to set MRS agar and incubated at the same temperature and duration as that of the faeces sample (Mami *et al.*, 2019; Marroki *et al.*, 2011). After 48 h incubation, colonies with small circular morphology, showing cream and off-white colour on MRS agar were selected for presumptive identification (Marroki *et al.*, 2011) according to Sharpe (1979). The selected lactic acid bacteria (LAB) isolates were characterized by morphological characteristics, Gram stain reaction and catalase test (Ann and Singh, 2018). Only isolates that were Gram positive, catalase negative and showing cream to off-white colour on MRS agar were selected and stored in 50% glycerol at -20ºC for molecular identification.

Isolation of bacterial pathogens: For isolation of pathogens, 1 g of faecal samples from rabbit was dissolved in 9 mL of normal saline and homogenized by vortexing. The mixtures were serially diluted and 1mL × 10^{-5} dilutions were plated out by the pour plate method in nutrient agar medium and incubated at a temperature of 37ºC for 24 h. The swab stick samples were also dipped into 9 mL sterile normal saline, homogenized and serially diluted. One milliliter (1 mL) of 10^{-4} dilution was introduced into sterile molten nutrient agar medium, mixed, poured into sterile Petri dish, allowed to solidify and incubated at 37ºC for 24 h. After incubation, the colonial growths on the plate were counted and recorded before they were purified by streaking unto an already solidified sterile nutrient agar in plates and incubated as above. Confirmatory identification of isolates was done by growing the pure colonies obtained on differential media such as mannitol salt agar (MSA), MacConkey agar and Eosin Methylene Blue Agar (EMB); and the inoculated media incubated as above. For further identification, Gram stain reaction was carried out to determine their morphology and structures. Biochemical tests including indole, citrate and oxidase tests were also carried out on the bacteria isolates.

Viable count of lactic acid bacteria and pathogens: One milliliter (1 mL) of 10^{-5} dilutions of overnight culture of presumptively identified lactic acid bacteria was added to sterile molten MRS agar (Oxoid, UK) and poured into sterile Petri dish. Inoculated agar in Petri dishes were left to set firmly before incubating at 37ºC for 48 h in a microaerophilic environment using the CampyGen (Oxoid). At the end of the incubation period, colonies formed by the lactic acid bacteria were counted based on differences in their morphology on MRS agar plates to determine the colony forming unit per gram (cfu/g) of faeces. One milliliter (1 mL) of 10^{-4} dilution of isolated pathogens was introduced into sterile molten nutrient agar medium, mixed, poured into sterile Petri dish, allowed to solidify and incubated at 37ºC for 24 h. Bacteria colonies formed were counted to determine the colony forming unit per gram (cfu/g) of faeces.

Molecular identification of LAB and pathogenic bacterial isolates: Deoxyribonucleic acid (DNA) of isolated strains was extracted by Accu Prep®Genomic DNA Extraction Kit (South Korea) following the manufacturer's instructions. The DNA extracted from the LAB isolates was used as the template in Polymerase Chain Reaction (PCR) amplification of the 16S rRNA gene with primers previously published and a modification of the PCR conditions described by Adeniyi *et al.* (2015). The PCR conditions were: 10 min of initial incubation at 95ºC, 15 sec of initial denaturation at 95ºC followed by 45 cycles of annealing at 55ºC for 30 sec, extension for 30 sec at 72ºC. This was followed by a single 7-min extension at 72ºC, and finally set on hold at 4˚C. The PCR products were purified, sequenced by GATC Biotech AG, Germany and the identity of the sequenced data obtained was compared with those held in Genbank database using the Basic Local Alignment Search Tool (BLAST) program.

Antibiotic susceptibility of isolated lactic acid bacteria: Antimicrobial susceptibility of lactic acid bacteria (LAB) isolated from rabbit faeces and fur to selected antibiotics was determined by disk diffusion method (Naser *et al.*, 2016). Twenty milliliters (20 mL) of MRS agar was poured into Petri dish and left to solidify. One hundred microlitre (100 µL) of LAB cultures equivalent to 0.5 McFarland standard (approximately 1 × 10^{8}) was spread on the surface of set agar by means of sterile swab stick. Antibiotic disk containing Ceftazidine-30 µg, Cefuroxine-30 µg, Gentamicin-10 µg, Ceftriaxone-30 µg, Erythromycin-5 µg, Cloxacillin-5 µg, Ofloxacin-5 µg and Augmentin®-30 µg was placed firmly on the dried surface of MRS agar using a sterile forceps and incubated at 37ºC for 24 h in a microaerophilic environment using CampyGen (Oxoid). Clear zones of growth inhibition around the antibiotic disks are indication of susceptibility of

the test organisms (LAB) to the selected antibiotics. Results were interpreted using the CLSI (2016) guidelines.

Antibiotic susceptibility of isolated bacteria: The bacteria pathogens isolated from rabbits (*Myroides gitamensis* and *Citrobacter rodentium*) were screened for susceptibility to eight (8) antibiotics by disk diffusion method. Eighteen milliliters (18 mL) of Mueller Hinton agar was poured into a sterile Petri dish and allowed to set. A bacterial lawn was accomplished by spreading of 1×10^8 of the pathogen cultures which is approximately equivalent to 0.5 McFarland standard by sterile swab stick. Antibiotic disk containing Ceftazidime-30 µg, Cefuroxime-30 µg, Gentamicin-10 µg, Ciprofloxacin-5 µg, Ofloxacin-5 µg, Augmentin® -30 µg, Nitrofurantoin-300 µg, and Ampicillin-10 µg was placed firmly on the dried surface of the solidified agar using a sterile forceps and incubated aerobically at 37ºCfor 24 h. Clear zones of growth inhibition around the antibiotic disks are indication of susceptibility of the test organisms to the selected antibiotics. The results were interpreted using the clinical laboratory standard institute guidelines (CLSI, 2016).

Antibacterial activity of viable cells of lactic acid bacteria and cell-free supernatant: The agar overlay (Hockett and Baltrus, 2017) and agar diffusion (Lawal *et al.*, 2014) methods were used to investigate the antibacterial activities of viable cells and cell-free supernatant (CFS) of LAB isolates, respectively, against pathogens isolated from the same habitat. Cell-free supernatant of the LAB was obtained by spinning overnight broth culture of LAB at 10,000 rpm for 10 min. The supernatant was carefully transferred into another sterile tube. Fifty microlitre (50 µL) of CFS was introduced into 6 mm agar well bored into inoculated agar in Petri dish. Test plates were incubated at 37ºC for 24 h. Clear zones of inhibition indicating susceptibility of test pathogens to the LAB isolates were measured in millimeter (mm).

RESULTS

Table 1:
Antimicrobial susceptibility profile of lactic acid bacteria (LAB) isolates from faeces of rabbit

LAB isolate	Antibiotic (diameter of zone of inhibition in mm)							
	CAZ	CRX	GEN	CTR	ERY	CXC	OFL	AUG
Lactobacillus plantarum 01	6,R	6,R	20,S	15,R	21,S	6,R	13,I	6,R
L. plantarum 02	6,R	6,R	26,S	6,R	31,S	6,R	6,R	6,R
L. plantarum 03	6,R	6,R	26,S	6,IR	31,S	6,R	6,R	6,R
L. plantarum 04	6,R	6,R	26,S	6,R	33,S	6,R	6,R	6,R
L. plantarum 05	6,R	6,R	15,S	6,R	18,S	6,R	6,R	22,S
L. plantarum 06	20,I	6,R	12,I	6,R	6,R	6,R	6,R	6,R
L. plantarum 07	12,R	6,R	15,S	13,R	18,S	6,R	6,R	6,R
L. plantarum 08	14,R	15,I	14,I	13,R	18,S	6,R	6,R	6,R
L. plantarum 09	13,R	6,R	13,I	14,R	17,S	6,R	6,R	20,S
L. plantarum 10	6,R	6,R	6,R	6,R	6,R	6,R	6,R	6,R
L. pentosus 11	13,R	6,R	15,S	6,R	20,S	6,R	6,R	24,S
L. fermentum 12	13,R	6,R	13,I	15,R	15,S	6,R	6,R	20,S
L. pentosus 13	6,R	6,R	13,I	6,R	20,S	6,R	6,R	23,S
L. pentosus 14	6,,R	6,R	6,R	6,R	26,S	6,R	19,S	30,S
L. pentosus 15	6,R	6,R	6,R	18,R	25,S	20,S	8,R	30,S
L. fermentum 16	6,R	6,R	20,S	20,I	29,S	6,R	6,R	26,S
L. pentosus 17	6,R	6,R	20,S	20,I	29,S	6,R	6,R	26,S
L. pentosus 18	6,R	6,R	40,S	6,R	40,S	6,R	6,R	31,S
L. pentosus 19	6,R	6,R	23,S	20,I	30,S	6,R	12,R	33,S
L. pentosus 20	6,R	6,R	34,S	6,R	37,S	6,R	6,R	33,S

Key: R-resistant, I- Intermediate, S-sensitive, Diameter of disc = 6 mm, CAZ-Ceftazidime 30 µg, CRX- Cefuroxime 30 µg, GEN-Gentamycin 10 µg, CTR- Ceftriaxone 30 µg, ERY: Erythromycin 5 µg, CXC- Cloxacillin 5 µg, OFL- Ofloxacin 5 µg, AUG- Augmentin 30 µg (CLSI, 2016).

Antibiotic susceptibility pattern of LAB and bacteria isolated from faeces of rabbits: Eighty (80) presumed lactic acid bacteria (LAB) were isolated from twenty-four (24) samples (12 faecal and 12 fur swab samples) collected from apparently healthy rabbits, out of which twenty (20) were identified and characterized as LAB belonging to three (3) species of Lactobacilli. The species are *Lactobacillus plantarum, L. pentosus* and *L. fermentum. Citrobacter rodentium* (Accession number: NR02865.1) and *Myroides gitamensis* (NR125560.1)] were also isolated from the same samples. Colony forming unit per gram (cfu/g) of faecal sample were $1.5 \times 10^5 – 2.94 \times 10^9$ and $2 \times 10^2 – 1.6 \times 10^{11}$ for the isolated LAB and pathogens, respectively. Antibiotic susceptibility assay for both the LAB and the pathogens in this study revealed varied resistant pattern to the various antibiotics used (Tables 1 & 2). Isolated LAB were resistant to five of the eight antibiotics (Table 1), having the highest resistance (95%) to Cefuroxime and Cloxacillin and least resistance (15%) to Gentamycin. *Lactobacillus plantarum* 10 had 100% resistance to the test antibiotics (Table 1). For the pathogens used in this experiment, *Citrobacter rodentium* showed resistance (100%) to all the eight antibiotics, while *Myroides gitamensis* had 62.5% resistance (Table 2).

Table 2:
Antimicrobial susceptibility profile of bacteria pathogens isolates from faeces of rabbit

Bacteria isolate	Antibiotic							
	CAZ	CRX	GEN	CPR	OFL	AUG	NIT	AMP
Myroides gitamensis	R	R	S	S	S	R	R	R
Citrobacter rodentium	R	R	R	R	R	R	R	R

Key: R-resistant, I- Intermediate, S-sensitivity, Ceftazidime (CAZ) 30 µg BP (R≤19, I=20-21, S≥22), Cefuroxime (CRX) 30 µg BP (R≤14, I=14-17, S≥18), Gentamicin (GEN) 10 µg BP (R≤13, I=13-14, S≥14), Ciprofloxacin (CPR) 5 µg BP (R≤16, I=16-20, S≥20), Ofloxacin (OFL) 5 µg BP (R≤13, I=13-15, S≥15), Augmentin (AUG) 30 µg BP, The resistance pattern is rare), Nitrofurantoin (NIT) 300 µg BP (R≤15, I=15-16, S≥16), Ampicillin (AMP) 10 µg BP (R≤14, I=14-16, S≥16), BP = Break point in reference to CLS1 Clinical Breakpoint (Whonet 5.6), 2016.

Table 3:
LAB isolates identification by 16S rRNA genes with percentage similarity

LAB isolate	Percentage (%) Accuracy
Lactobacillus plantarum 01	100
L. plantarum 02	99
L. plantarum 03	99
L. plantarum 04	99
L. plantarum 05	99
L. plantarum 06	99
L. plantarum 07	99
L. plantarum 08	99
L. plantarum 09	100
L. plantarum 10	97
L. pentosus 11	99
L. fermentum 12	99
L. pentosus 13	99
L. pentosus 14	99
L. pentosus 15	99
L. fermentum 16	99
L. pentosus 17	99
L. pentosus 18	99
L. pentosus 19	99
L. pentosus 20	99

Identification of lactic acid bacteria (LAB) isolated from faeces of rabbits: The lactic acid bacteria (LAB) isolates were presumptively identified by morphological characteristic, Gram stain reaction and biochemical (catalase) test. Isolates (colonies) that presented with the following features: small circular morphology, raised elevation, cream/off white in colour, smooth and having entire margin, Gram-positive and catalase negative (data not shown) were selected for further study. The identity of the isolates was confirmed by sequencing the 16S rRNA genes. 16S rRNA genes of twenty (20) of the LAB isolates were amplified as confirmed by agarose gel electrophoresis following polymerase chain reaction (PCR). The species of lactic acid bacteria identified were *Lactobacillus plantarum, Lactobacillus pentosus* and *Lactobacillus fermentum;* the percentage homology are as presented in Table 3. Ten (10 i.e., 50%) of the isolates belong to the species plantarum, eight (8 i.e., 40%) of the isolates belong to the species pentosus, while two (2 i.e., 10%) of the isolates belong to the species fermentum. *Lactobacillus plantarum* had the highest frequency of occurrence, closely followed by *Lactobacillus pentosus,* while *Lactobacillus fermentum* had the lowest frequency of occurrence.

Table 4:
Inhibition of *Myroides gitamensis* (AP20) and *Citrobacter rodentium* (BP13) by LAB viable cells. Radius of zone of inhibition (mm) ± SEM

LAB isolate	Radius Zone of inhibition (mm)	
	Myroides gitamensis (AP20)	*Citrobacter rodentium* (BP13)
Lactobacillus plantarum 01	27 ± 1.0	10 ± 0.0
L. plantarum 02	20 ± 1.0	32 ± 1.0
L. plantarum 03	25 ± 0.0	12 ± 0.0
L. plantarum 04	27 ± 1.0	17 ± 1.0
L. plantarum 05	29 ± 1.0	35 ± 0.5
L. plantarum 06	30 ± 1.0	23 ± 1.0
L. plantarum 07	25 ± 1.0	30 ± 1.0
L. plantarum 08	30 ± 1.0	40 ± 0.0
L. plantarum 09	30 ± 1.0	40 ± 0.0
L. plantarum 10	30 ± 1.0	20 ± 0.5
L. pentosus 11	25 ± 0.5	29 ± 1.0
L. fermentum 12	27 ± 0.0	34 ± 1.0
L. pentosus 13	25 ± 0.0	36 ± 0.0
L. pentosus 14	22 ± 1.0	19 ± 1.0
L. pentosus 15	18 ± 1.0	18 ± 1.0
L. fermentum 16	30 ± 1.0	35 ± 0.5
L. pentosus 17	20 ± 0.0	30 ± 1.0
L. pentosus 18	25 ± 1.0	38 ± 1.0
L. pentosus 19	27 ± 0.5	30 ± 1.0
L. pentosus 20	25 ± 0.5	30 ± 0.0

Antimicrobial activities of viable LAB and cell free supernatant of LAB isolates against bacteria isolates: Viable cells of the LAB were evaluated for their antibacterial activity against *Citrobacter rodentium* and *Myroides gitamensis* the pathogens isolated from the same source as the LAB. All of the isolates had varying antibacterial activity against the test pathogens as shown by the diameter of the zones of inhibition (Table 4). *Lactobacillus plantarum* exhibited the highest antibacterial activity against *Citrobacter rodentium* (radius zone of inhibition = 40 + 0.0 mm). *Lactobacillus plantarum* and *L. fermentum* both demonstrated the highest activity (radius zone of inhibition = 30 + 1.0 mm)

in *Myroides gitamensis. Lactobacillus pentosus* had a low antibacterial activity against *Myroides gitamensis* with radius zone of inhibition of 18 + 1.0 mm (Table 4). The cell free supernatant (CFS) of the LAB was also evaluated for antimicrobial activity in *Myroides gitamensis* and *Citrobacter rodentium* isolated from the faeces of rabbits. The results as shown in table 5 revealed antagonistic activity of the CFS from all the isolates against the *Myroides gitamensis* with highest diameter zone of inhibition of 20 + 1.0 mm whereas against *Citrobacter rodentium*, CFS did not give hundred per cent activity (Table 5).

Table 5:
Inhibition of *Myroides gitamensis* (AP20) and *Citrobacter rodentium* (BP13) by LAB cell free supernatant. Diameter of zone of inhibition (mm) ± SEM

LAB isolate	Diameter Zone of inhibition (mm)	
	Myroides gitamensis (AP20)	*Citrobacter rodentium* (BP13)
Lactobacillus plantarum 01	18 ± 1.0	12 ± 0.0
L. plantarum 02	15 ± 1.0	15 ± 0.0
L. plantarum 03	12 ± 0.0	13 ± 1.0
L. plantarum 04	18 ± 0.0	12 ± 1.0
L. plantarum 05	15 ± 1.0	12 ± 0.5
L. plantarum 06	15 ± 0.5	NZI
L. plantarum 07	20 ± 1.0	13 ± 1.0
L. plantarum 08	18 ± 1.0	09 ± 0.5
L. plantarum 09	10 ± 0.0	14 ± 1.0
L. plantarum 10	15 ± 1.0	15 ± 1.0
L. pentosus 11	15 ± 1.0	15 ± 0.0
L. fermentum 12	18 ± 0.0	NZI
L. pentosus 13	20 ± 0.0	10 ± 1.0
L. pentosus 14	17 ± 1.0	16 ± 1.0
L. pentosus 15	18 ± 0.5	NZI
L. fermentum 16	10 ± 1.0	10 ± 0.5
L. pentosus 17	20 ± 1.0	14 ± 1.0
L. pentosus 18	08 ± 0.0	NZI
L. pentosus 19	15 ± 1.0	17 ± 0.5
L. pentosus 20	10 ± 1.0	13 ± 1.0

Key: NZI- No zone of inhibition

DISCUSSION

The interaction between man and animals either as food animals or pet animals has been in existence for a long time. This interaction exposes man to zoonotic pathogens necessitating the use of antibiotics most of which are known to kill both the pathogenic bacteria and beneficial bacteria.

In this study, the antibacterial activity of lactic acid bacteria (LAB) isolated from the faeces and fur of rabbits against two pathogenic microorganisms isolated from the same sources was investigated. Fur swabs and faeces samples were used in order to draw a link of the spread of infections between the rabbit, its environment and human beings. This is similar to the work done by Minna *et al.* (2003) who reported the interaction between probiotic lactic acid bacteria and canine enteric pathogens. Tinrat *et al.* (2011) also reported the antibacterial activity of six (6) out of sixty (60) LAB isolated from faeces against *Salmonella typhi* and *S. typhimurium*. In another study by Adeniyi *et al.* (2015), the antimicrobial activities of cow's intestinal lactic acid bacteria (LAB) against enteric commensals were reported. More recently, Pohilko and Kravchenko (2018) reported the antagonistic effects of Lactobacilli species isolated from rabbits against *Salmonella* species.

The LAB and the pathogens were subjected to some selected antibiotics to determine their susceptibility pattern using the disk diffusion methods as described (Naser *et al.,* 2016). The LAB showed a high resistance to five of the eight antibiotics used in the study, having the highest resistance (95%) to cefuroxime and cloxacillin and least resistance (15%) to gentamycin. This finding suggests that the activity of the isolated LAB will not be inhibited by the test antibiotics should they be used as therapeutic agents in the animal in this study. It also means that the stability of isolated LAB will not be affected by the test antibiotics particularly when probiotics are administered after treatment with antibiotic(s) (Kilic *et al.,* 2005; Xu *et al.,* 2008).

On the other hand, the isolated pathogenic bacteria-*Citrobacter rodentium* was resistant (100%) to all the eight antibiotics, while *Myroides gitamensis* had 62.5% resistance. These bacteria are multidrug-resistant having shown resistance to more than three different classes of antibiotics. *Citrobacter rodentium* is pathogenic in mice especially laboratory mice with suckling mice being the most susceptible and causes transmissible murine colonic hyperplasia (TMCH). This disease though self-limiting is characterized by hyperproliferation of the epithelial cell of the descending colon (Gart, 2011). The occurrence of *Citrobacter rodentium* in rabbit as found in this study could have resulted from contamination of rabbits' feed by faecal droppings of free-range mice and accidental contamination of feed by the personnel from unwashed hands. Since rabbits are known to eat their faecal droppings, a process known as coprophagy, it is possible for the animals to have eaten faecal droppings of mice in their feeds thereby ingesting *Citrobacter rodentium*.

In a study by Thomas *et al.* (2018) the antibiogram profile of *Myroides gitamensis*- MG554743 revealed that the isolate was resistant to all the antibiotics (10 antibiotics comprising ofloxacin, ciprofloxacin, ampicillin, ceftazidime, norfloxacin, ceftriaxone, levofloxacin, chloramphenicol, gatifloxacin and cefotaxime) used in the study. The resistance of *M. odoratimimus* strain PR63039 to sixteen (16) different antibiotics was also reported by Hu *et al.* (2016). These reports show that Myroides species are multidrug-resistant bacteria. The genus Myroides (family Flavobacteriaceae) are non-fermenting, Gram-negative, aerobic, and non-motile bacteria [Kim *et al.,* 2012] previously isolated only from clinical sources, aquatic environment, grey mullet's gut, and flesh flies [Hu *et al.,* 2017]. Infection with this bacterium requires long term combined administration of antibiotics. These pathogens *Citrobacter rodentium* and *Myroides gitamensis* were susceptible to the isolated LAB. The antibacterial activity of the viable cells and the cell-free supernatants (CFS) of the LAB revealed that the viable LAB cells had better antibacterial activity against the tested pathogens with radius of zone of inhibition ranging between 10 + 0.0 - 40 + 0.0 mm. The antibacterial activity so demonstrated as seen in this study is better and significant considering that the pathogens in this study were multi-drug resistant. These findings justify the use of probiotics as safe alternative to antibiotics. Lactic acid bacteria (LAB) do not only prevent pathogenic micro-

organisms' invasion but also produce antimicrobial substances while maintaining microbial balance (Linares *et al.,* 2017).

Isolated LAB as well as the pathogenic bacteria which had been phenotypically identified were subjected to molecular identification a very accurate and reliable method for the identification of bacteria including LAB (Amor *et al.,* 2007). Twenty (20) LAB were identified which is distributed between three species of Lactobacillus, these species are *Lactobacillus plantarum* (10), *Lactobacillus pentosus* (8) *and Lactobacillus fermentum* (2). *Lactobacillus plantarum* which had fifty per cent (50%) occurrence of the total LAB identified, is more dominant in the faeces and fur swab samples of rabbit. In a study by Pohilko and Kravchenko (2018), *Lactobacillus fermentum* alongside other Lactobacilli species was isolated from the gastrointestinal tract of rabbit unlike Linaje *et al.* (2004) who reported that Lactobacilli were not isolated from neither intestinal content nor faeces of rabbits in their study. The most commonly used probiotics are the Lactobacilli and they are known to produce high concentrations of organic acids due to their metabolic activities (Linares *et al.,* 2017). The occurrence of these LAB with pathogenic bacteria in the same environment suggests that the LAB were able to effectively prevent the multiplication of these pathogens in the gut thus, the pathogens were not able to cause any infection in the animal. This may account for the apparent healthy status of the animals used in this study.

In conclusion, twenty (20) lactic acid bacteria (LAB) distributed between three species of Lactobacillus- *Lactobacillus plantarum, Lactobacillus pentosus* and *Lactobacillus fermentum* were isolated from the faeces and fur of rabbits, identified and characterized. They were investigated for antibacterial activity against two pathogenic microorganisms- *Citrobacter rodentium* and *Myroides gitamensis* isolated from the same source. The occurrence of these LAB with pathogenic bacteria in the same environment suggests that the LAB were able to effectively prevent the multiplication of these pathogens in the gut hence, the inability of the pathogens to cause any infection in the animal. Antibacterial activity of whole viable LAB cells was significantly high compared to that of the cell free supernatant. The antibacterial activity demonstrated as seen in this study justifies the use of probiotics as safe alternative to antibiotics.

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