Journal of Advances in Biology & Biotechnology

24(5): 21-25, 2021; Article no.JABB.69143
ISSN: 2394-1081

Antibiotic Resistant Profile of Lactic Acid Bacteria Isolated from Swine and Poultry Faeces in Umuahia Metropolis

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Authors' contributions

This work was carried out in collaboration between both authors. Author RCOM designed the study, wrote the protocol, managed the literature searches, and wrote the first draft of the manuscript. Author OGN, managed the analyses of the study. Both authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JABB/2021/v24i530214
Editor(s):
(1) Dr. Fernando José Cebola Lidon, Universidade Nova de Lisboa, Portugal.
Reviewers:
(1) Anil Deka, Assam Agricultural University (AAU), India.
(2) M. Abareethan, Periyar University, India.
(3) Wanglar Alphonsa Moyon, South East Manipur College (SEMCO), India.
Complete Peer review History: http://www.sdiarticle4.com/review-history/69143

Short Research Article

Received 27 March 2021
Accepted 02 June 2021
Published 18 June 2021

ABSTRACT

Aim: This study's aim was to determine the antibiotics resistant profile of lactic acid bacteria isolated from poultry and swine faeces.
Study design: Faecal samples from swine and birds were randomly collected from livestock and poultry farms located in Umuahia metropolis, Abia State.
Place and duration of study: Department of microbiology, Michael Okpara University of Agriculture Umudike, between January 2019 to August 2019.
Methodology: A total of 12 faecal samples, 6 each from swines and birds were examined for the presence of lactic acid bacteria using Deman Rogosa Sharpe agar supplemented with 0.3% $CaCO_3$ (w/v). Isolates were identified based on their physiological and biochemical characteristics. Antibiotic susceptibility was carried out using disk diffusion method.
Results: Of the 12 faecal samples examined, all were positive for lactic acid bacteria, with counts ranging from 1.74 – 2.36 x 10^6 in swine and 1.52 – 2.08 x 10^6 in birds. Total of 14 strains that belong to three genera; *Lactobacillus*, *Lactococcus* and *Streptococcus* were isolated, genus *Lactobacillus*

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occurred highest 8(57.1%). The isolates showed multidrug resistance and exhibited high rate of resistance to Augmentin (100%), Ceftazidime (100%), Cefotaxime (92.9%), Erythromycin (85.7%), Ceftriaxone (71.4%) and Azithromycin (71.4%).
Conclusion: The antibiotic resistance pattern of the isolated lactic acid bacteria is a clear indication that most animal farmers are misusing antibiotics. Therefore, animal farmers should be advised on antibiotic application safety measures.

Keywords: Antibiotic resistance; lactic acid bacteria; swine; birds.

1. INTRODUCTION

Lactic acid bacteria (LAB) produce lactic acid as their major end product during the fermentation of most fermentable sugars especially glucose [1]. Lactic acid bacteria species are taxonomically diverse with numerous genera, despite the frequent changes in LAB taxonomic classification, the widely accepted genera include; *Carnobacterium, Enterococcus, Lactobacillus, Lactococcus, Leuconostoc, Oenococcus, Pediococcus, Streptococcus, Tetragenococcus, Vagococcus* and *Weissella*. Some authors include the genus *Bifidobacterium* because of its probiotic role, although it belongs to a different phylogenetic group [1, 2].

Antibiotic resistant bacteria have the ability to resist the activities of naturally occurring, semi-synthetic or synthetic compounds unfavorable to their survival [3]. The excessive use and abuse of antibiotic led to the emergence of antibiotic resistance. Antibiotic resistant pathogenic bacteria pose a great potential threat to human health, especially in an immuno-compromised system [4]. Antibiotic resistant maybe intrinsic or acquired, and most acquired are through gene transfer. In 2002, the presence of identical resistant gene in bacterial isolates of different hosts was reported by Scott, [5].

In most developed countries the use of antibiotic as animal growth promoter has been prohibited [6]. Thus, various alternatives have been explored to replace antibiotics as growth promoters. Lactic acid bacteria are probiotics and have been embraced by many as alternative to chemical growth promoters in livestock animals [7]. Lactic acid bacteria could still serve as antibiotics resistant genes reservoir which might be transferred to pathogenic bacteria. The presence of antibiotics resistant LAB in animal faeces is an indication that the intestinal LAB is carrying a resistant gene which might be passed to human through food chain [6,7,8]. Also, the search for green fertilizers has encouraged the use of animal faeces as fertilizers. The presence of antibiotic resistant LAB in animal faeces poses a threat to crop productivity as resistant gene might be transferred to plant pathogens. As stated by Sundin, [9] antibiotic resistant in plant – pathogenic bacteria is a problem to the pathosystems. However, the information on antibiotic resistant LAB from animal faeces in Nigeria is very limited. Hence the aim of this work was to determine the prevalence of antibiotic resistant in Lactic acid bacteria isolated from birds and swine faeces in Umuahia metropolis, Abia state.

2. MATERIAL AND METHODS

2.1 Collection of Samples

A total of Twelve (12) samples of animal faeces from 12 different farms, 6 from poultry farms and 6 from piggery farm were collected from Umuahia metropolis in Abia state. Samples were stored in sterile plastic containers and taken to the laboratory for microbiological analysis.

2.2 Isolation of Lactic Acid Bacteria

Using the serial dilution technique, 1g of each faecal sample was homogenized in a 9 ml physiological saline. From 10^{-3} dilutions0.1 ml eachwas inoculated onto molten (45^{o}C) DeMan Rogosa Sharpe (MRS) agar supplemented with 0.3% $CaCO_3$ (w/v) and allowed to solidify. The inoculated plates were incubated at 30°C for 48hrs in an anaerobic condition. Colonies showing clear zones were counted and selected for further studies.

2.3 Identification of Lactic Acid Bacteria

The pure isolates were identified on the basis of their physiological characteristics (Gram reaction, cultural characteristics) and biochemical characteristics (catalase tests, coagulase tests, Voges-proskaeurtest, indole test, citrate test, oxidase test) and sugar utilization test were done according to the method of [10].

2.4 Antibiotic Sensitivity Assay

The antibiotic susceptibility of the LAB isolates were tested against the following antibiotics; Cefoxitin (30 µg), Ceftriaxone (45 µg), Cefexime(5µg), Levofloxacin (5 µg), Gentamicin (10 µg), Ciprofloxacin (5 µg), Imipenem(10 µg), Cefuroxime (30 µg), Azithromycin (15 µg) and Cefotaxime (25 µg), using Kirby Bauer antibiotics disk method.Cell pellet of the test LAB was suspended into peptone water. The turbidity of the suspension was adjusted to 0.5 Mc farland standard, then spread on MRS agar. The Antibiotic disk was placed on the inoculated plate using forceps. Incubation was carried out under anaerobic condition for 48h at 30^{o}C. Thereafter, the diameter of the zone of inhibition was measured [11].

3. RESULTS AND DISCUSSION

3.1 Total Lactic Acid Bacteria Counts of Swine and Bird Fecal Samples

The total viable count of LAB obtained ranged from 1.74×10^6 - 2.41×10^6in swine and 1.54×10^6 - 2.08×10^6 in birds as shown in (Table 1). A high total viable lactic acid bacteria count was obtained in this study, this is in agreement with the finding of Puphan et al. [12] which reported a high LAB count of 5.15 x 10^7 – 1.25 x 10^{12} from animal faeces. The high total viable LAB count obtained from animal faeces might be attributed to the fact that most farms use commercial probiotics as growth promoters [13].

Table 1. Total viable lactic acid bacterial counts of swine and poultry fecal samples

Samples	Total LAB count (cfu/g) 10^6	Log of Total LAB count
P_1	2.08	6.32
P_2	1.78	6.25
P_3	1.72	6.24
P_4	1.52	6.18
P_5	1.69	6.23
P_6	1.54	6.19
S_1	2.36	6.37
S_2	1.88	6.27
S_3	2.41	6.38
S_4	2.04	6.31
S_5	1.74	6.24
S_6	1.81	6.26

Cfu/g = Colony forming unit per gram
S = Swine, P = poultry

3.2 Percentage Occurrence of the Lactic Acid Bacteria Isolates

The morphological and biochemical characteristics of Lactic acid Bacteria isolates, revealed the presence of 14 strains that are affiliated to 3 genera; *Lactobacillus*, *Lactococcus* and *Streptococcus*. The isolation of lactic acid bacteria from the swine and poultry faeces is a reflection of the strains of LAB on the intestinal tract of birds and swine. The Lactic acid bacterial isolates from this study is similar to the findings from study conducted by Lin et al. [7] isolated *Lactobacillus* and *Streptococcus* from the intestinal tract of pigs. Also Shazali et al. [6] reported *Lactobacillus, Lactococcus* among the lactic acid bacteria genera isolated from faeces samples of broiler.

The occurrence rate of the Lactic acid bacteria isolates showed that *Lactobacillus* spp was the most frequently occurring genus with a percentage occurrence of 8(57.1%), followed by *Lactococcus* with a percentage occurrence of 4(28.6%), and *Streptococcus* spp with the least percentage occurrence of 2(14.3%) (Table 2). This is in agreement with the findings of the study conducted by Shazali*et al*. [6] who demonstrated that different LAB species were isolated from different wet market and the most common LAB genus isolated from chicken faecal sample was *Lactobacillus* followed by *Lactococcus*. However, the distribution of Lactic acid bacteria in the samples showed that *Lactobacillus* (62.5%) was frequently isolated from bird and *Lactococcus* (100%) from swine. *Lactococcus* was not isolated from bird faeces. The high occurrence rate of genus *Lactobacillus* might be explained by the fact that most commercial probiotics contains *Lactobacillus*.

3.3 Antibiotic Sensitivity Profile of the Lactic Acid Bacterial Isolates

The antibiotic resistant pattern of the LAB revealed that the isolates obtained from faecal samples showed low resistance to levofloxacin (7.1%), followed by ofloxacin (14.3%), ciprofloxacin (21.4%) and gentamicin (28.6%). The Lactic acid bacteria isolates were highly resistant to augmentin and ceftazidime with percentage resistance of (100%), this is followed by cefotaxime (92.9%), erythromycin (85.7%), cetriaxone (71.4%) and azithromycin (71.4%) (Table 3). These results were in agreement with the previous report by Erginkaya et al. [14] on

Table 2. Distribution of Lactic acid Bacteria in the samples

Isolate	Swine (n=6) No. of isolate (%)	Bird (n= 6) No. of isolate (%)	Occurrence rate in both samples (%)
Lactobacillus spp	3(37.5)	5(62.5)	57.1
Lactococcus spp	4(100)	0(00)	28.6
Streptococcus spp	1(50)	1(50)	14.3
Total No. (%)	8(57.1)	6(42.9)	100

Table 3. Antibiotic resistant profile of lactic acid bacteria

Genera	Number of isolate	AUG (%)	OFX (%)	CTX (%)	CXM (%)	CRO (%)	LBC (%)	CIP (%)	ERY (%)	GEN (%)	AZN (%)
Lactobacillus spp	8	100	25	100	100	62.5	12.5	25	75	37.5	75
Lactococcus spp	4	100	0.0	75	100	75	0.0	25	100	25	50
Streptococcus spp	2	100	0.0	100	100	100	0.0	0.0	100	0.0	100
Total	14	100	14.3	92,9	100	71.4	7.1	21.4	85.7	28.6	71.4

***KEY**: AUG = augumentin, OFX = ofloxacin, CFX = Cefoxatime, CXM = Ceftazidime, CRO = Ceftriaxone, LBC = Levofloxacin, CIP = Ciprofloxacin, ERY = Erythromycin, GEN = Gentamicin, AZN = Azithromycin.*

LAB isolates of animal origin being resistant to erythromycin (100%), and β-lactam antibiotics, they also found the LAB isolates susceptible to gentamicin (70%). One can say the high resistance of LAB to β-lactam group of antibiotics is intrinsic, since most LAB lack peptioglycan layer which is the major target of these antibiotics [14]. Also resistance to erythromycin might be as a result of the easily transferrable Erm B resistant gene common in LAB as stated by Yenizey et al. [4]. This result is contrary to the report of Wang et al. [8] that *Streptococcus* spp were highly susceptible and also *Lactobacillus* spp were recorded to be highly resistant to gentamicin (100%). Also, Contrary to this report was the result of Shazali et al. [6] where *Lactobacillus* spp were most sensitive to erythromycin, ampicillin and resistant to gentamicin and ciprofloxacin. Lactic acid bacteria antibiotics resistance might be intrinsic or extrinsic (acquired), the acquired resistance is complicated as resistant gene can be horizontally transferred to pathogens.

4. CONCLUSION

Fourteen strains of LAB affiliated to three genera were isolated from faecal samples of poultry and swines. Each strain of LAB displayed different level of antibiotic resistant. These results revealed that LAB may have acquired the resistant gene from other gut micro flora through horizontal gene transfer. The antibiotic resistance development can be attributed to long term use or misuse of antibiotics as growth promoters and therapeutics. Therefore, there should be regulation in the livestock industry on the use of antibiotics. The use of LAB by farmers as a growth promoter should be monitored and regulated to ensure that only probiotics from known source are used.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Giraffa G. Overview of the ecology and biodiversity of the lactic acid bacteria: Hotzapfel WH, Wood BJB editors. Lactic acid bacteria biodiversity and taxonomy. Ist ed. London: John Wiley; 2014.
2. Khalid K. An overview of lactic acid bacteria. Int. J. Bioscience. 2011;211(1):1-13.
3. World Health Organization. Antimicrobial resistance Bad bug, no drugs as antibiotic discovery stagnates a public health crisis advance a review. Official J. Australian Soc. Microbiol. 2007; 28:152-167.
4. Yenizey M, Alvarez C, Alquicira EP. Antibiotic resistance in lactic acid bacteria: Antimicrobial resistance a global threat. Yashwant Kumar. Intechopen. 2018.Accessed 15 March 2019.
5. Scott KP. . The role of conjugative transposons in spreading antibiotic resistance between bacteria that inhabit the gastrointestinal tract.Cell Mol. Life Sci.2002;59:2071–2082.
6. Shazali N, Foo HL, Loh TC, Choe DW, Rahim RA. Prevalence of antibiotic

resistance in lactic acid bacteria isolated from the faeces of broiler chicken in Malaysia. Gut Pathol. 2014;6:1-9.

7. Lin CF, Lin MY, Lin CN, Chiou MT, Cheng JW, Yang KC, Wu MC. Potential probiotic of lactic acid bacteria strains isolated from intestinal tracts of pigs and faeces of Dogs with antibacterial activity against multidrug resistant pathogenic bacteria. Arch. Microbiol. 2020;202:1849 – 1860.
8. Wang. K, Zhang H, Feng J, Ma L, Fuente-Nunez C, Wang S, Lu X. Antibiotic resistance of lactic acid bacteria isolated from dairy products in Tianjin China. J Agric. Food Res.. 2019;1(1):100006.
9. Sundin W, Wang N. Antibiotic resistance in plant pathogenic bacteria. Annu. Rev. Phytopathol. 2018;25(56):161 – 180.
10. Harrigan WF, McCane ME. Laboratory methods in food and dairy microbiology 3rd Ed. New York, san Fransisco. Academic Press; 1998.
11. Bauer AW, Kirb MWM, Sherris JC, Turck M. Antibiotic Susceptibility testing by standard disk method. Am. J. Clin. Pathol. 1996;45:493 – 496.
12. Puphan K, and Sornplang P, Uriyapongson S, Naranukraw C. Screening of lactic acid bacteria as potential probiotic in beef cattle. Pak. J. Nutr. 2015;8:478–479.
13. Dowarah R, Verma AK, Agarwal N. The use of *Lactobacillus* as alternative growth promoter in pigs. Anim. Nutr. 2016;3(1):1-6.
14. Erǵinkaya Z, Turhan EU, Tatli D. Determination of antibiotic resistant of lactic acid bacteria isolated from Turkish fermented dairy product. Iran. J Vet. Res. 2018;19(1):53- 56.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sdiarticle4.com/review-history/69143