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 $1.74 \times 10^6$ in swine and $1.52 \times 10^6$ in birds. Total of 14 strains that belong to three genera, *Lactobacillus*, *Lactococcus* and *Streptococcus* were isolated, genus *Lactobacillus*
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 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 each was inoculated onto molten (45°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-proskauer test, indole test, citrate test, oxidase test) and sugar utilization test were done according to the method of [10].

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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°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 - 2.41×10^6 in 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

<table>
<thead>
<tr>
<th>Samples</th>
<th>Total LAB count (cfu/g) 10^6</th>
<th>Log of Total LAB count</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1</td>
<td>2.08</td>
<td>6.32</td>
</tr>
<tr>
<td>P2</td>
<td>1.78</td>
<td>6.25</td>
</tr>
<tr>
<td>P3</td>
<td>1.72</td>
<td>6.24</td>
</tr>
<tr>
<td>P4</td>
<td>1.52</td>
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</tr>
<tr>
<td>P5</td>
<td>1.69</td>
<td>6.23</td>
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<tr>
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<tr>
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<td>S5</td>
<td>1.74</td>
<td>6.24</td>
</tr>
<tr>
<td>S6</td>
<td>1.81</td>
<td>6.26</td>
</tr>
</tbody>
</table>

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 cefazidime with percentage resistance of (100%), this is followed by cefotaxime (92.9%), erythromycin (85.7%), ceftriaxone (71.4%) and azithromycin (71.4%) (Table 3). These results were in agreement with the previous report by Erginkaya et al. [14] on
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 peptidoglycan 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.

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6. Shazali N, Foo HL, Loh TC, Choe DW, Rahim RA. Prevalence of antibiotic

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