



Preliminary Screening of Bacteria Isolated from Insects Living in Poultry for Antibiotic Production

**Ifeoma Geraldine Okudo^{1*}, Chinelo Ursula Umedum¹,
Stephen Nnaemeka Ezekwueche¹ and Chibuzo Christian Uba²**

¹Department of Microbiology, Chukwuemeka Odumegwu Ojukwu University, Anambra State, Nigeria.

²Department of Microbiology, Paul University, Awka, Anambra State, Nigeria.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/MRJI/2020/v30i230198

Editor(s):

(1) Lachhman Das Singla, Guru Angad Dev Veterinary and Animal Sciences University, India.

Reviewers:

(1) Itodo, Sunday Ewaoche, Niger Delta University, Nigeria.

(2) Satadal Das, Peerless Hospital & B. K. Roy Research Centre, India.

Complete Peer review History: <http://www.sdiarticle4.com/review-history/55726>

Original Research Article

Received 03 February 2020

Accepted 10 April 2020

Published 16 April 2020

ABSTRACT

Aim: This present study was conducted to isolate antibiotic producing bacteria from insects living in poultry.

Place and Duration of Study: Insects living in poultry were collected from poultry farms within Onitsha metropolis in Anambra State between April 2018 and September 2018.

Methodology: The gut of one hundred insects; (*Musca domestica* and *Alphitobius diaperinus*) were analyzed. The insects were dissected and emulsified in 10ml of peptone water. The dilutions were cultured on Nutrient agar and Blood agar for 24 h. The bacterial isolates were characterized using molecular identification. The DNA was extracted from the identified isolates and analyzed by 16S rRNA. In preliminary screening, the isolates were inoculated into Muller Hinton agar using agar plug. The promising isolate showing antagonism was subjected to submerged fermentation method and the secondary metabolites were extracted. Screening of the secondary metabolites extract was done using agar well diffusion. The minimum inhibitory concentration (MIC) of the secondary metabolite was determined using broth dilution method at different concentrations. The inhibitory activity of the organism was checked against four bacteria namely; *Bacillus subtilis*, *Salmonella serovar typhi*, *Escherichia coli* and *Staphylococcus aureus*.

*Corresponding author: E-mail: okudogeraldine@gmail.com;

Results: The sequence analysis revealed the strains to be *Lysinibacillus macroides*, *Paealcaligenes hermetiae*, *Bordetella flabilis*, *Bacillus aerophilus*, *Klebsiella variicola*. *Lysinibacillus macroides* showed antagonism against the test bacteria during the preliminary test. After fermentation, the secondary metabolite extracts from *Lysinibacillus macroides* exhibited antibacterial activities against *Salmonella Serovar Typhi*, *Staphylococcus aureus* and *Bacillus subtilis* at different concentrations.

Conclusion: The extracts from bacterial isolate; *Lysinibacillus macroides* exhibited antibacterial activities against *Bacillus subtilis*, *Salmonella serovar typhi* and *Staphylococcus aureus*. The extracts may serve as a new drug molecule produced from natural source when purified.

Keywords: Antagonism; antibiotic production; bacteria; Minimum Inhibitory Concentration (MIC); preliminary screening; secondary metabolites.

1. INTRODUCTION

Insects are considered the most successful group of animals in terms of both diversity and survivability in various ecological niche. The micro flora and gut flora of an insect contains many important bacterial species of biological importance as well as fungi and other pathogenic microorganism [1]. So far, numerous pathogenic bacteria including *Salmonella spp.*, *Shigella spp.*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Escherichia coli* have been isolated from insects [2].

Houseflies (*Musca domestica*) are the most important insect pest associated with poultry, where the accumulated organic waste and the favourable environmental conditions often promote rapid development of large population. The role of the housefly in the transmission of pathogens and gastrointestinal diseases such as Shigellosis, Salmonellosis, Cholera and yaws has been firmly established [3]. Structurally, the fly is well adapted for picking up pathogens. It is therefore not surprising that as many as 6×10^6 bacteria have been found on the exterior surface of a single feeding fly and more than 100 species of pathogenic organisms have been isolated from the digestive tract of flies. Pathogenic bacteria remain alive in houseflies for an appreciable time [3,4].

The litter beetles (also called the lesser mealworm beetle) or *Alphitobius diaperinus* is also common insect associated with commercial and sometimes backyard poultry where they can be abundant in the poultry litter or manure. Livestock (Poultry) get infected when pathogenic organism passes to the susceptible animal through feeding [4]. It is known widely as a pest of stored food grain products such as flour and of poultry rearing facilities. It is a vector of many kinds of animal pathogens and reservoirs for a

number of serious poultry disease agents and can also act as intermediate hosts for caecal nematodes, tapeworms and protozoa [5]. In addition, they can transmit a number of food-borne pathogen such as *Escherichia coli* and *Salmonella typhi* and have been recently implicated in the transmission of *Campylobacter*.

As the insect is a vast repository of microorganisms, many of which remain undetected, it is a potential source of many species with the ability to produce new antibiotics [6]. Thousands of antibiotics have already been discovered from other sources. However, only a very small proportion of known antibiotics are used clinically because the others are either too toxic, or show other undesirable properties. For example, by 1978, nearly 5000 antibiotics had been described of which only 95 were being used for therapy [7].

The initial discovery of antibiotics occurred in the late 1920s and 1930s and the first useful antibiotic was penicillin (a beta-lactam antibiotic). Penicillin was "accidentally" discovered by Alexander Fleming. He noticed that a fungus (*Penicillium*) was producing a substance that prevented the growth of bacteria on a discarded agar medium plate. Howard Florey and Ernst chain took this discovery to the next step which led to the successful application of Penicillin in the treatment of infections. All three were awarded the Nobel Prize in 1945 for their work with Penicillin [8].

Selman Waxman adapted Fleming's observations into a screening method to find other microorganisms that produced similar antibacterial substances. His screening technique involved growing potential antibiotic producing microorganisms on the same medium as a pathogen and looking for inhibition of growth of the pathogen by the other microorganism. This technique led to the discovery of Streptomycin

from *Streptomyces griseus* and a flood of other antibiotics in the 1940s to 1960s [9].

The discoveries of new types of antibiotics have slowed in the 50 years since the golden age of antibiotic discovery. This is an unfortunate event in that during this same period of time, antibiotic resistance among bacterial strains has increased, thus making it more difficult to treat bacterial infections [10]. Resistance to antibiotics can be modified by a genetic mutation or by a bacterial enzyme, so the target no longer interacts with the antibiotic. An antibiotic can also be modified or completely destroyed by a bacterial enzyme so it no longer can function (for example, some bacteria synthesize beta lactamases that cleave beta-lactam antibiotics to neutralize their activities). An antibiotic can also be prevented from entering the bacterial cell or if the antibiotic gets into the cell, it is pumped back out. Examples of specific pathogens that have become multiple resistant to several antibiotics include methicillin resistant *Staphylococcus aureus* (MRSA) and extensively drug resistant (XDR) *Mycobacterium tuberculosis*. Needless to say, new methods of antibiotic discovery must be pursued in order to keep up with the evolving rise in bacterial resistance [11].

The present work was therefore carried out to screen bacterial isolates from insects living in poultry for antagonistic effects on test bacterial isolates and subsequent production and extraction of secondary metabolites.

2. MATERIALS AND METHODS

2.1 Sample Collection

Insects were collected from different local poultry farms around Ochanja market, Onitsha, Anambra State Nigeria. Two different insects (*Alphitobius diaperinus* and *Musca domestica*) were used for the study and ten of each insects was collected from each poultry farms. Adult houseflies (*Musca domestica*) were collected using insect net, the flies were baited with 10% sugar solution on a glass slide while a transparent sterile universal container with an opening and a lid was used to trap the litter beetles (*Alphitobius diaperinus*) as described in another study by Yang et al. [12].

After the sample collection, all the samples were labelled accordingly. The samples were transported alive to the laboratory and immobilized at a temperature of 10°C for 5 mins

to 10 mins. Captured insects were identified by species using morphological characteristics.

2.2 Surface Sterilization of Insects

In laboratory, each insect was washed with 70% ethanol in a sterile conical flask (To decontaminate external surfaces as 70% ethanol is bactericidal), transferred to sterilized flasks, and allowed to dry at room temperature under sterile conditions [13].

2.3 Dissection of the Insects

The insect was then transferred and fixed on to a disinfected dissection board and the abdomen was exposed with the aid of sterile razor blade and forceps. It was then placed in 10 ml of sterile peptone water in a test tube and emulsified. The samples were left at room temperature for 2 h before being processed [13].

2.4 Sample Processing

Ten fold serial dilutions were carried out using sterile test tubes. The mixtures were serially diluted using aseptic technique. 10^{-5} , 10^{-6} and 10^{-7} were plated on Nutrient Agar and Blood Agar. The culture plates were incubated for 24 h as described by Amanda and his colleagues [14].

2.5 Isolation of Bacteria

The culture plates were then observed for growth and all the distinct colonies were subcultured until pure colonies were obtained. Pure colonies were then maintained on the appropriate agar slant and kept in the refrigerator for further use [15].

2.6 Identification and Classification of Bacterial Isolates

Themolecular identification was carried out using 16S rRNA sequencing which involves DNA extraction, PCR amplification and sequencing process [16].

DNA extraction: Genomic DNA extraction from bacteria isolates was carried out using the DNeasy Blood and Tissue Extraction Kit (Qiagen, USA) following the protocol provided by the manufacturer.

PCR amplification: The isolated DNA was used as template for PCR using primer specific to bacterial 16S rRNA sequence. The forward and reverse primers were applied. PCR primer 27F

5¹(AGA GTT TGA TCM TCG CTC AG) 3 and 1492R 5¹ (TAC GGY TAC CTT GTT ACG ACT T) 3I were used for the amplification. The PCR reaction mixture with a final volume of 50 µl was set up accordingly: 5 µl Buffer (10x), 3 µl MgCl₂ (2.5 mM), 1 µl dntps, 1.5 µl tag polymerase, 1.5 µl forward Primer, 1.5 µl reverse primer, 3 µl DNA template and 34.7 µl distilled water. DNA template of 1.5 ul, 1 ul of both forward and reverse primers each and 21.5 ul of nuclease free water in a PCR tube were added in that order. The PCR was performed in a Biorad PCR with initial denaturation of 95°C for 2-5 min followed by 30 cycles of denaturation at 94°C for 30s. Annealing and elongation was done at 52°C for 30 sec and 72°C for 2 min respectively in 45 cycles. Final extension was at 72°C for 10 min. The PCR products were further analyzed by electrophoresis using 1.5% (w/v) agarose gel. The amplified PCR were gel purified and were used for sequencing [17].

Sequencing reactions were performed in the DNA Engine Tetrad 2 Peltier Thermal Cycler (BIORAD) using the ABI Big Dye^R Terminator v3. 1 cycle sequencing kit (Macrogen) following the protocols supplied by manufacturer. Single Pass sequencing was performed on each template using universal primer. The fluorescent – labeled fragments were purified from the unincorporated terminators with the Big Dye X Terminator^R purification kit (Macrogen). In addition, the nucleotide BLAST (NCBI) program compared the homology of the nucleotide sequence in related bacteria.

2.7 Bacterial Organisms used for the Screening

The test organism (*Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli* and *Salmonella SerovarTyphi*) were obtained from Microbiology Department of Paul University, Awka, Anambra State. The four test organisms were identified using confirmatory biochemical tests before use.

2.8 Primary Screening of Isolates for Antagonism

An agar culture of the bacterial isolates were made on Nutrient agar plate by spreading on the plate and incubated for 24 h at 37°C. In this method, 0.5 Mcfarland standard of 24 h broth culture of the test organisms (*Bacillus Subtilis*, *Escherichia coli*, *Salmonella SerovarTyphi* and *Staphylococcus aureus*) were prepared. After

incubation, an agar plug of 6mm was cut aseptically from culture plate with a cork borer and deposited on the surface of Mueller Hinton agar previously inoculated with the test microorganisms. These were incubated at 37°C for 24 h. The antimicrobial activity of the microbial secreted molecules was detected by the appearance of the inhibition zone around the agar plug after the incubation period [18].

2.9 Secondary Metabolite Extraction

Bacteria isolates from the insects with potential antimicrobial activity indicated by inhibition zone were selected for submerged fermentation and subsequent extraction of antimicrobial compounds. The selected antagonistic bacteria isolates were inoculated into peptone water medium enriched with 15.6 g of starch, 2.0 g of NH₄SO₃, 4.0 g of Yeast extract, 1.98 g of MgSO₄.7H₂O, 1 g of K₂HPO₄, 2 g of CaCO₃, 0.004 g of FeSO₄.7H₂O in 1000 ml of distilled water at pH 7.2 ± 0.2 as described by Jose et al. 2013 [19]. 150 ml of medium was dispensed into Erlenmeyer flask, sterilized at 121°C for 15 mins and cooled. At room temperature, each of the medium was inoculated with 2 ml 24 h broth culture of the bacteria isolates. These were incubated at 30°C for 8 days. The culture medium was occasionally shaken throughout the incubation period. At the end of the incubation period, secondary metabolites were extracted by adding equal volume of ethyl acetate (1:1v/v) into the fermentation media and then shaken vigorously for 1 h. The solvent phase was separated from aqueous phase by using a separating funnel. To obtain the extract, the solvent phase was concentrated using a water bath temperature at 60° [20].

2.10 Determination of the Antibiotic Activity of the Extracts

The extracts of the fermented medium were evaluated for antibacterial activity using the Agar well diffusion method. In this method, 0.5 Mcfarland and Standard of 24h broth culture of (*Escherichia coli*, *Staphylococcus aureus*, *Salmonella Serovar Typhi* and *Bacillus subtilis*) were prepared and inoculated on the surface of Mueller Hinton agar using a sterile cotton swab. Agar wells were prepared using a sterile cork borer (6mm in diameter). A volume of 100 ul of the extracts was carefully dispensed into each well and allowed to diffuse for 2 h and the plates were incubated at 37°C for 24 h. Amoxicillin was used as positive control. After 24 h of incubation, each culture plates were observed for zones of

inhibition around each well which was measured and recorded [21].

2.11 Minimum Inhibitory Concentration (MIC) Determination

To measure the MIC value, various concentration of the stock was prepared according to the method of Pallota et al. [22] modified by Agu et al. [23]. The minimum inhibitory concentration (MIC) was determined by the broth dilution method. Overnight culture of the clinical isolates were diluted to 0.5 McFarland Standard. Different test tubes containing nutrient broth which contained 20%, 40%, 60% and 80% of the extracts were inoculated with the test isolates i.e 20% contains 0.2 ml of the extract and 0.8 ml of the nutrient broth. The tubes were incubated at 37°C for 24 h. The tubes incubated without bacterial extracts were set up as control. Thereafter, the incubated test tubes were then subcultured onto sterile freshly prepared plates and incubated for 24 h. At the end of the incubation period, the plates were examined for growth. The plates with the lowest count was recorded as the MIC. The MIC is defined as the lowest concentration required to arrest the growth of the microorganism at the end of 24 h or 48 h of incubation [22,23].

2.12 Statistical Analysis

The prevalence rate of the bacterial isolates was ascertain using the T-test with the level of significance set at $P < 0.05$.

3. RESULTS

3.1 Identification of Bacterial Isolates from Insects Living in Poultry

Based on the molecular identification that was carried out on the isolates using 16S rRNA sequencing, the majority of the isolates were observed to be *Lysinibacillus macroides*,

Paealcaligenes hermetiae, *Bordetella flabilis*, *Bacillus aerophilus*, *Klebsiella variicola*.

The frequency of occurrence of the bacterial isolates isolated from the insects in the poultry farms is shown in Fig. 1. The frequency of *Klebsiella variicola* has the highest percentage 41.7%, *Bordetella flabilis* 27.8%, *Bacillus aerophilus* 16.7%, *Lysinibacillus macroides* 8.3% and *Paealcaligenes hermetiae* 5.6% (P value = 0.04).

Fig. 2 shows the phylogenetic tree of the bacterial isolate; *Lysinibacillus macroides*. The type strain is LMG 18474T (=DSM 54T=ATCC12905T). The result shows that *Lysinibacillus macroides* is genomically distinct from its close relatives *Lysinibacillus boronitolerans* and *Lysinibacillus pakistanensis*.

3.2 Antagonistic Effect of Bacterial Isolates on Test Organisms

Preliminary screening of bacterial isolates for antibiotic production against test bacterial revealed that one isolate was able to exhibit inhibition zone on *Bacillus subtilis*, *Staphylococcus aureus* and *Salmonella* Serovar *Typhias* in Fig. 3.

Antimicrobial evaluation of the extracts/secondary metabolites of bacterial isolates showed that only one bacterial isolate had potential antimicrobial activity against the test bacteria. *Lysinibacillus macroides* extract inhibited the growth of *Bacillus subtilis*, *Staphylococcus aureus* and *Salmonella* Serovar *Typhi* as in Table 1.

Different concentrations; 20%, 40%, 60% and 80% of the crude extracts of *Lysinibacillus macroides* were used to calculate the Minimum Inhibitory Concentration (MIC) of the extracts against the clinical isolates. *Lysinibacillus macroides* showed the MIC at 60% for *Bacillus subtilis* and at 80% for *Salmonella typhi* and *Staphylococcus aureus* as shown in Table 2.

Table 1. Screening of the extracts /secondary metabolites of bacteria Isolates by agar well diffusion method

Isolates	Zone of Inhibition (mm)		
	LME	AMX	Well Diameter
<i>Bacillus subtilis</i>	18.0 ± 0.5	22.0 ± 0.0	6.00
<i>Staphylococcus aureus</i>	15.0 ± 1.2	12.0 ± 0.0	6.00
<i>Escherichia coli</i>	0.0 ± 0.0	10.0 ± 0.0	6.00
<i>Salmonella typhi</i>	20.0 ± 0.5	25.0 ± 0.0	6.00

LME - *Lysinibacillus macroides* extract, AMX - Amoxicillin

Table 2. Minimum inhibitory concentration of *Lysinibacillus macroides* against the test isolates

Concentration (%)	L M E (Minimum inhibitory concentration.)		
	<i>Bacillus subtilis</i>	<i>Salmonella typhi</i>	<i>Staphylococcus aureus</i>
20	+	+	+
40	+	+	+
60	-	+	+
80	-	-	-
A M X(mg/ml)	-	-	-

LME- *Lysinibacillus macroides* extract, AMX- Amoxicillin; + means presence of growth; - means no growth

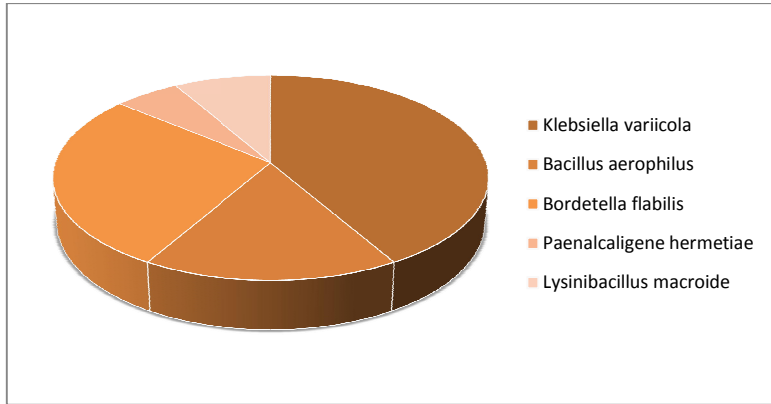


Fig. 1. Percentage frequency of occurrence of bacteria Isolates from insects living in poultry

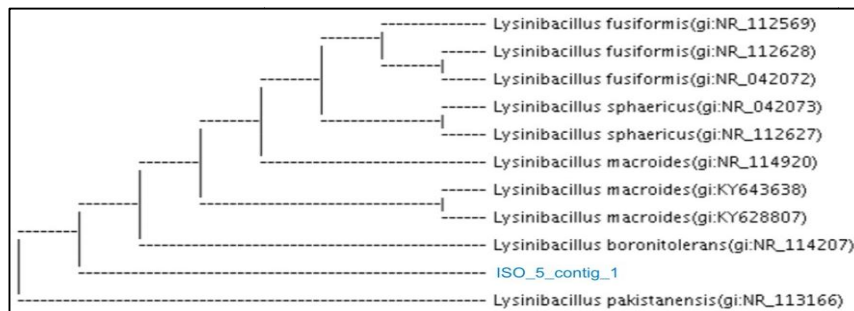


Fig. 2. Phylogenetic tree of *Lysinibacillus macroides*

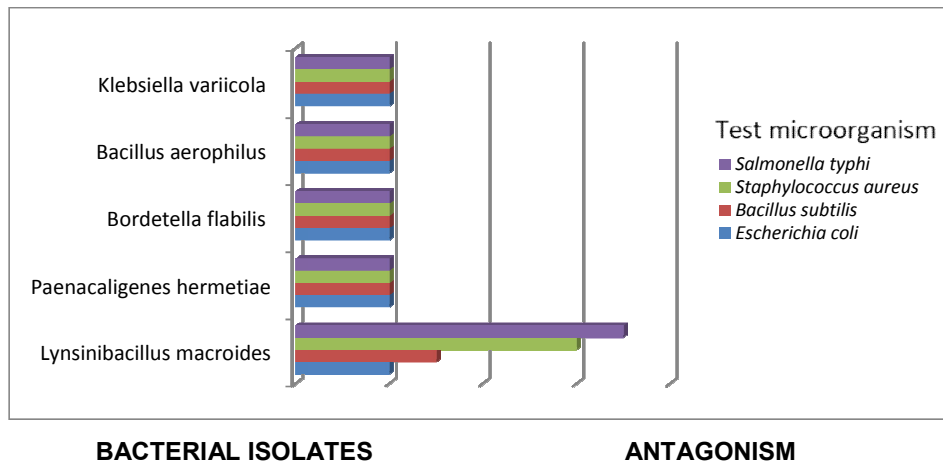


Fig. 3. Bar chart showing antagonistic effects of bacterial isolates on test isolates

4. DISCUSSION

In search of new antibiotics, relatively simple and rapid methods have been developed for screening microorganisms for antibiotic producing ability. Insect living in poultry are not commonly employed in the isolation of antibiotic producing organism but was chosen for this research work. The detection of these antagonistic substances revealed interesting properties that justify its importance and its study on the potential application in biological control of pathogenic microorganisms. Many microorganisms have been evaluated for the production of antimicrobial substance however the high cost and low yields have been the main problem for its industrial production [24].

In the present study, insects (*Muscadomestica* and *Alphitobius diaperinus*) living in poultry were selected randomly from different poultry farms within Onitsha metropolis for isolation of antibiotics /secondary metabolite producing bacteria. Previous studies showed that selection of different potential areas such as soil rhizosphere, seawater were important places for isolation of different types of potent antibiotic / secondary metabolite producing bacteria [25]. The gut of the insects were dissected and the samples were collected and analyzed. Based on the recent study as many as 6×10^6 bacteria have been found on the exterior surface of a single feeding fly and more than 100 species of pathogenic organisms have been isolated from the digestive tract of flies.

Five bacteria were isolated and the identity of the isolate were determined based on the colonial description, morphological characteristics and biochemical studies of the bacterial isolates from insects living in poultry. Microscopic examination of the isolates after Gram staining showed that all the isolates were Gram -ve bacteria. In addition, more modern techniques were used involving DNA studies. Information's about the unknown bacteria is elucidated by sequencing its entire genome and analyzing it for specific gene sequences. Therefore molecular characterization has also been carried out in the present investigation, to identify the frequently isolated species of bacteria with the help of 16S rRNA gene sequencing. Based on the molecular characterization that was carried out, the sequence analysis revealed the strains to be *Lysinibacillus macroides*, *Paenalcaligenes hermetiae*, *Bordetella flabilis*, *Bacillus aerophilus* and *Klebsiella varicola*. The 16S rRNA gene

sequence of strain LMG 18474T with ascension no. KY643638.1 showed 99% similarity to that of *Lysinibacillus macroides*. The 16S rRNA gene sequence of strain KBL 009 with ascension no. NR109523.1 showed 100% similarity to that of *Paenalcaligenes hermetiae*. In the recent years much attention has been given to the housefly as a potential mechanical vector of disease transmitting agent. Study conducted by Nazni et al. 2005 showed that in Malaysia, several bacterial species were isolated from the housefly *Musca domestica*. His study indicated that more variety of bacteria species were isolated from houseflies in the poultry farm compared to the dumping ground. Some of the bacterial species they isolated such as *Bacillus* sp., *Klebsiella* sp., *Enterobacter* sp., *Escherichia* sp., *Acinetobacter* sp., *Proteus* sp., were also obtained in this present study from flies living in poultry.

The primary screening using agar plug method indicated that one (20%) out of five poultry isolates showed potential antimicrobial activity against the test bacteria as shown in Fig.3. Observation of clear inhibition zones around the wells on the inoculated plates is an indication of antimicrobial activities of secondary metabolite extracted from isolated bacteria. Previous studies have shown that several extracts from bacteria exhibited antimicrobial activity. Of the 44 Actinomycetes isolated by Bhagwan et al. 2007, five isolates showed antibacterial activity and were identified as *Streptomyces* species while 45.9% of the Streptomycin isolates from soil produced antibacterial activity [26].

The bacteria producing inhibition zone against the clinical strains were selected for further characterization of their antibacterial activity. In the Secondary screening, the antibacterial substance production of the extract of the bacterial isolate was further tested by agar well diffusion method. Inhibitory activity against the clinical isolates was also detected for one poultry isolates; *Lysinibacillus macroides* while no antibacterial activity against the clinical isolates was shown by the other poultry isolates; *Klebsiella variicola*, *Bordetella flabilis*, *Bacillus aerophilus* and *Paenalcaligenes hermetiae*. The secondary metabolite from *Lysinibacillus macroides* showed lower antimicrobial activity against *Bacillus subtilis*, *Salmonella* Serovar Typhi and *Staphylococcus aureus* in comparison with standard antibiotic (amoxicillin 20 ug/ml). The extract of *Lysinibacillus macroides* showed the minimum inhibitory concentration at 60% for *Bacillus subtilis*, 80% for *Staphylococcus aureus*

and *Salmonella typhi*. It has therefore shown that the bacterial isolate has an inhibitory effect against both Gram positive and Gram negative bacteria. This study has demonstrated that the production of antibacterial substances is widespread among different bacterial strains in different locations. The poultry isolates obtained in the present study showed antibacterial activity against both Gram positive and Gram negative clinical isolates. Although insects that are living in poultry have been found to contain multidrug resistant bacteria but does not necessary prevent such bacteria from producing antibiotics. Previous studies showed that bacteria producing antibiotics had been isolated from marine bacteria and identified as *Lysinibacillus sphaericus* [27]. The results conform to many reviews of literature that *Lysinibacillus* spp. are known to produce bioactive secondary metabolites.

Understanding antibiosis at the phylogenetic level may allow a more focused search for antibiotics that are active against a bacterial species or group. Such an understanding may also help to devise strategies for pathogen control in poultry environment. Current assay for antimicrobial activity are inadequate because some antibiotic producing bacterial may require the presence of an inducer compound produced in the presence of another bacterial species. These finding have important implication for the discovery of novel antimicrobial compounds from poultry bacterial and may allow the development of new method for screening novel compounds active against multi-drug resistant bacteria.

5. CONCLUSION

This research work showed that the extract from *Lysinibacillus macroides* may possess antibacterial activities against both Gram positive bacteria; *Staphylococcus aureus* and Gram negative bacteria; *Bacillus subtilis*, *Salmonella* Serovar *Typhi*.

The findings of this study indicated that insects that were living in poultry may provide rich source for the discovery of novel antimicrobial antibiotics. Importantly, *Lysinibacillus macroides* displayed a potential benefit in controlling infection caused by bacteria which may decrease medical burden thereby improving effectiveness of drugs produced from natural sources. Further purification and structural elucidation are recommended to validate the novelty, quality and commercial value of the antibiotics.

ETHICAL APPROVAL

There were ethical issues in the collection of the samples.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Nardi JB, Mackie RI, Dawson JO. Could microbial symbionts of arthropod guts. Contribute significantly to nitrogen fixation in terrestrial ecosystems. *Journal of Insect Physiology*. 2000;48:75–763.
2. Thyssen PJ, Moretti T, Uleta MT, Riberiro OB. The role of insects (Blattodea, Diptera and Hymenoptera) as possible mechanical vectors of helminthes in the domiciliary and peridomiciliary environment. *Cadernos de saudePublica Impact Factor Journal*. 2004;20:1096–1102.
3. Nazni WA, Seleena B, Jeffrey J, Rogyah TAR, Sofian MA. Bacteria fauna from the housefly, *Musca domestica*. *Journal of Tropical Biomedicine*. 2005;22(2):225–231.
4. BarnesM, Vallancourt JP, Gross WB. *Diseases of poultry*. Lava State University Press, Amessia USA; 2003.
5. Faraldo AC, Sa-Nunes A, Faceioli LH, Del Bel EA, Lello A. Synthase activity in tissues of the blowfly *Chrysomya megacephala*. *Biocell*. 2007;31(2):205–211.
6. Krishnan M, Bharathiraja C, Pandiarajan J. Insect gut microbiome an unexploited reserve for biotechnological application. *Asian Pacific Journal of Tropical Biomedicine*.2014;4:16–21.
7. Ahmed RN, Sani A, Ajjolakewu A, Folake B. Soil screening for antibiotic producing microorganisms. *Advances in Environmental Biology*. 2013;7(1):7–11.
8. Berdy J. Bioactive microbial metabolites. *Journal of Antibiotics*. 2005;58:1–26.
9. Williams ST, Locci R, Beswick A, Kurboke D.I, Kuznetsov VD, Lemonnior FJ, Long PF. Detection and identification of novel actinomycetes. *Research in Microbiology*. 1993;144(8):650-656.
10. Okudoh VI. Isolation and identification of antibiotic producing microorganisms from natural habitats in the Kwazulu natal midlanda. *Microbiology*. 2001;3–26.

11. Ravi C, Jeyashree A, Renuka DK. Antimicrobial peptides from insects: An overview. *Research Journal of Biotechnology*. 2011;2(5):1-7.
12. Yang L, Yu Y, Feng Z, Xuejun F, Wei Z, Dairong Q. Multi-drug resistant gram negative enteric bacteria isolated from flies. *South East Asian Journal of Tropical Medical Public Health*. 2013;44(6):988-996.
13. Bagde US, Gopi U, Prasad R. Isolation and characterization of gut associated microbes in cockroach. *African Journal of Microbiology Research*. 2013;7(19):2034-2039.
14. Amanda S, Motta F, Cladera-Olivera E, Brandelli A. Screening for antimicrobial activity among bacteria isolated from the Amazon basin. *Brazilian Journal of Microbiology*. 2004;35:307-310.
15. Bushra U, Nuzhat A, Farzana K, Edwards DH. Isolation and characterization of pseudomonas strain that inhibit growth of indigenous and clinical isolate. *The Internet Journal of Microbiology*. 2006; 2(2):62-71.
16. Janda JM, Abbott SL. 16s rRN A gene sequencing for bacterial identification in the diagnostic laboratory: Pluses, perils and pitfalls. *Journal of Clinical Microbiology*. 2007;45:2761-27264.
17. Elijah AI, Atanda OO, Popoola AR, Uzochukwu SVA. Molecular characterization and potential of bacterial species associated with cassava waste. *Nigerian Food Journal*. 2014;32(2):56-65.
18. Balouiri M, Sadiki M, Ibsouda SK. Methods for in vitro evaluating antimicrobial activity: A review. *Journal of Pharmaceutical Analysis*. 2016;6:71-79.
19. Jose PA, Sivakala KK, Jebakumar SRD. Formulation and statistical optimization of culture medium for improved production of antimicrobial compound by *Streptomyces sp.* *International Journal of Microbiology*. 2013;1-9.
20. Gebreyohannes G, Moges F, Sahile S, Raja N. Isolation and characterization of potential antibiotic producing actinomycetes from water and sediments of lake Tana, Ethiopia. *Asian Pacific Journal of Tropical Biomedicine*. 2013;3(6):426-435.
21. Illic SB, Konstantinovic SS, Todorovic ZB, Lazic ML, Veljkovic N. Characterization and antimicrobial activity of the bioactive metabolites in *Streptomyces isolated*. *Microbiology*. 2007;76(4):480-487.
22. Pallota RC, Ribeiro MS, De Lima Machado ME. Determination of the minimum inhibitory concentration of four medicaments used as intracanal medication. *Australian Endodontics Journal*. 2007;33:107-111.
23. Agu KC, Igweoha CA, Umeh CN. Antimicrobial activity of the ethanolic and petroleum ether extracts of tangerine seed on selected bacteria. *International Journal of Agriculture and Bioscience*. 2013; 2(1):22-24.
24. Vastrad BM, Neelagund SE. *International Journal of Pharmaceutical Sciences and Drug Research*. 2011;3(2):104-111.
25. Abo-Shadi M, Sidkey NM, Al-Mutrafy AM. Antimicrobial agent producing microbes from some soils' rhizosphere. *Journal of American Science*. 2010;6(10):915-925.
26. Nurettin S. Investigation of antimicrobial activity of some *Streptomyces* isolates. *Turkish Journal of Biology*. 2002;27:79-84.
27. Shanmugaraju V, Arun P, Rengaramanujam J, Senthilprabhu S. Isolation of bacteria from seacoasts and screening for its antibacterial substance production. *International Journal of Current Microbiology and Applied Sciences*. 2013; 2(8):347-358.

© 2020 Okudo et al.; This is an Open Access article distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Peer-review history:

The peer review history for this paper can be accessed here:
<http://www.sdiarticle4.com/review-history/55726>