



Isolation and Identification of Antibiotic Producing Bacteria from Soil Samples of Abattoir in Lapai, Niger State, Nigeria

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Authors' contributions

This work was carried out in collaboration among all authors. Authors EIU and ZJK planned and designed the research. Authors EIU and ARA wrote the proposal writing, discussion/choice of the protocol, performed the laboratory work, interpreted the results and wrote the manuscript for publication. Authors ZJK and AH did the literature researches, discussion/choice of the protocol, proposal writing proof reading and guided the laboratory work. All authors read, made scientific contributions and approved the final manuscript.

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ABSTRACT

Antimicrobial agents or antibiotics are the most significant commercially available and utilized secondary metabolites, which are highly produced by the soil microbes (bacteria and fungi) and found to be effective and broad spectrum. Microbes produce metabolic products (antimicrobial agents) through the process called antibiosis. Majority of the classes of antibiotics used are derivatives of animals (microbes) and floras (plants). But currently, the microbial resistance is at the top gear which requires more effort to come up with novel structure, effectual, toxic free and reasonable cost of new antimicrobial products against microbial infections. In the present study, a

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trial was made to isolate, identify and characterize the antibiotic producing bacteria from the soil samples collected from different sites of abattoir in Lapai, using the standard microbiological techniques. A total of nine (9) bacterial of both groups (Gram positive and negative) which includes *Corynebacterium diphtheriae*, *Bacillus cereus*, *Escherichia coli*, *Enterobacter aerogene*, *Clostridium* specie, *Bacillus subtilis*, *Pseudomonas* species, *Staphylococcus aureus* and *Klebsiella pneumoniae* were isolated in the course of the study. *Pseudomonas* species was the most frequently isolated bacteria (33.33%) while the rest of the isolates were 8.33% across the five different sampling sites. These isolates were further screened against some pathogenic microbes viz, *Salmonella* sp, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Candida albicans*. Three of the bacterial isolates isolated from the abattoir were found to exhibits antimicrobial activity against two pathogenic bacteria used in this study. This study indicates that some of the soil microorganisms could be an interesting source of antimicrobial bioactive substances.

Keywords: Bacteria; antibiotics; soil; antimicrobials; biosynthesizing; inhibition.

1. INTRODUCTION

There is an increasing numbers of drug-resistant strains, particularly the acquired multi-drug resistant strains which is becoming rapidly growing concern and leads to serious public health problems worldwide [1]. This is as a result of misuse and over prescription of antibiotics which has affected our ability to treat patients empirically [2,3]. Many bacteria have developed resistance against these common antibiotics through methods such as a reduced in cell wall permeability, efflux pump, and hydrolysis of the β -lactam ring by beta-lactamases. The production of metallo-beta-lactamases is the most important technique by which pathogenic bacteria becomes resistant to the present non beta-lactam antibiotics [4]. These have prompted to the necessity for urgent search and development of new pharmaceuticals to combat infectious ailments and curtail antimicrobial resistance [5].

Antibiotics are natural substance of biological, synthetic or semi-synthetic origin [6] and continue to play a crucial role in the development of tissue culture techniques and basic screenings, primarily in biochemistry, molecular biology, microbiology and genetics (including genetic engineering) and to a lesser extent, pharmacology and organic chemistry [7]. These days, researchers are searching for an array of microbes in order to improve the number of discoveries in pharmaceutical industries. Therefore, this finding will raise high possibility of identifying bacteria strain from soil samples which might be of biochemical and pharmacological virtues.

Soil is a complex and very diverse environment providing versatile source of antibiotic producing

organisms [8]. Microorganisms constitute an inexhaustible reservoir of compounds with pharmacological, physiological, medical or agricultural applications [9-11]. Most antibiotic used today are isolated from soil bacteria and recounted for their antibiotic production. *Bacillus* species being the common soil bacteria have been found limiting the growth of the other organisms because of their resistant endospore formed and production of vital antibiotics like bacteriocin. It is advisable to screen antibiotic producing bacteria as they are easy to isolate, culture, maintain and to increase the value of their strains [12]. However, these microbial populations in the soil depends on the various factors like soil type, water activity, oxygen, pressure, temperature, salt concentration, carbon sources, pH and other factors. Recent analyses have shown that screening of soil for antimicrobial activities have been carried out in many parts of the world [13].

Many pharmaceutically important antibiotics have been identified in the past from this bioresource, e.g., vancomycin produced by *Streptomyces orientalis* isolated from a soil sample from Borneo [14], kanamycin produced by a soil bacterium *Streptomyces kanamyceticus* [15] and erythromycin first isolated in 1952 from the soil bacterium *Saccharopolyspora erythraea* [16]. Bacterial genera reported so far as a bioresource with a high chance to detect compounds of interest are Actinomycetes [17], Bacilli [18] and *Pseudomonas* [19,20].

This research work was undertaken with an effort to isolate, identify and further characterize antibiotic producing bacteria from different soil sample of abattoir in Lapai metropolis, Niger State, Nigeria. Also to assess the inhibitory property of the isolated bacteria against some human pathogenic microbes.

2. METHODOLOGY

2.1 Study Area and Period

The study area was Lapai local government area in Niger State adjoining the Federal Capital Territory, Abuja. Its headquarter is in Lapai town, located along A124 highway at latitude 090°-02'N and longitude 060°-34'E. As a result of the establishment of high institution; Ibrahim Badamasi Babangida University in Lapai, several developments have taken place in Lapai and has also lead to the gradual increase of slaughtering areas (abattoir). The study was carried out from August to October, 2018.

2.2 Sample Collection

Soil samples were collected from five different sites of the abattoir (North, South, West, East and Centre). The samples were taken from 5-8 cm depth into dry, clean, and sterile black polythene bags, tightly sealed and immediately transported to the Department of Microbiology, Ibrahim Badamasi Babangida University, Lapai, Niger State, Nigeria where the entire research work was carried out.

2.3 Sample Preparation

One gram of each soil sample from the 5 sites was accurately weighed. Six test tubes were taken; 9mL of distilled water was dispensed into each test tube. The tubes were subjected to sterilization in an autoclave at 121°C for 15 minutes. One gram each of the soil samples was suspended in 9ml of distilled water and vortex for 2-3 minutes to obtain homogenous mixture and carried out serial dilution. From the stock culture, a volume of 1mL was aseptically transferred to the next tube and serial dilution continues down to tube 6 [21].

2.4 Isolation of Antibiotic Producing Bacteria

Nutrient agar was prepared in line with manufacturer's directives and sterilized in an autoclave at 121°C for 15 minutes and allowed to cool to 45°C. The media was poured into petri dishes and allowed to gel under aseptic condition. A volume of 1 mL of suspension from 10⁻⁴ serially diluted tubes were taken and spread evenly with sterile L-shaped glass rod and was aerobically incubated at 37°C for 24h [22].

2.5 Sub Culturing of the Bacteria

After overnight incubation, the plates were closely observed. A loopful of distinct colonies were aseptically picked and further sub-cultured on freshly prepared nutrient agar for 24 h at 37°C to obtain pure cultures for further analysis.

2.6 Identification of Bacteria Isolates

The identification of the isolated bacterial species was done by Gram staining technique and biochemical tests and comparing the results with standard description given in Bergey's Manual of Systematic Bacteriology [8]. The biochemical tests performed were Indole test, Catalase test, Citrate Utilization test, Methyl Red test, Coagulase test, Urease test, Endospore staining, Carbohydrate fermentation test (glucose, fructose, sucrose and lactose) [23,24].

2.7 Antimicrobial Assay

The antimicrobial assay was carried against four isolates which were sourced from Microbiology Department, IBB University, Lapai. The tested organisms include *Salmonella* specie, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Candida albicans*.

2.8 Antimicrobial Screening

Antibacterial activity was determined by agar well diffusion method using Muller-Hinton Agar plates. Cell concentration of 0.5 McFarland standard of all the tested organisms were inoculated in Muller-Hinton agar plates using sterilized cotton swabs. The wells were bored by sterile cork borer, and 100 µl (0.1 mL) of the isolated isolates were introduced into the wells. The plates were incubated at 37°C for 24 h. Zones of inhibition were observed and measured in vertical and horizontal direction [5].

3. RESULTS

This study was carried to screen the antibiotic producing bacteria collected from soil sample of five (5) different sites of the abattoir in Lapai, using standard microbiological techniques. The table below shows the different sites and various activities carried out in the sites, dilution factors, number of colonies, bacteria count (cfu/mL) and the logarithm of the bacteria count (microbial load). The microbial load presence in the five different studied points of abattoir in Lapai varied

from point to point. The presence of bacterial in the soils was recorded high in the North compared to others Table 1.

A total of nine (9) bacterial isolates of both groups were identified in the course of this study after culturing and characterization. The below table shows the characteristics of the bacteria isolates as were seen in their respective shapes during microscopy and also shows their biochemical characteristics and their action on different test. The isolates include *Corynebacterium diphtheriae*, *Bacillus cereus*, *Escherichia coli*, *Enterobacter aerogene*, *Clostridium* specie, *Bacillus subtilis*, *Pseudomonas* species, *Staphylococcus aureus* and *Klebsiella pneumoniae* Table 2.

The table below shows the percentage of occurrence of bacteria isolates in five different sampling sites (North, South, West, East and Centre) in Lapai abattoir. It was observed that *Pseudomonas* species was the most frequently isolated bacteria (33.33%) while the rest of the isolates were 8.33% across the five different sampling sites Table 3.

The native soil microbes are believed to have high tendency of antimicrobial potential. The identified cultures of bacteria were then checked for antibiotic production activity against three pathogenic strains of bacteria which includes *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Salmonella* specie and a fungi, *Candida albicans* using agar well diffusion method. Under normal growth condition, the zones of inhibition were observed against Gram negative organisms (*P. aeruginosa* and *K. pneumoniae*) Table 4.

4. DISCUSSION

The study of soil microbes is significant in order to know the microbes that present especially the

antibiotic producing ones. Soil harbor wide range of microflora, it is possible to find out new species and strains with higher possibility to produce new antimicrobial substances [25]. The number and types of microbes can be use to ascertain the degree of microbial load in the soil. This research work shows the type of bacteria isolated from soil samples of abattoir. Soil sample from abattoir in Lapai was chosen for analysis in this study base on the likelihood of the presence of microorganisms that have not previously been studied by other researchers. Keeping in mind the above-mentioned point, this study dealt with isolation of new strains of bacteria from soils and screened for antimicrobial activity against some pathogenic microbes.

In these present findings, the sampling sites shows different bacteria count. The highest and lowest bacteria counts were observed at the North and South region of the sampling site with 2.16×10^6 and 8.8×10^5 cfu/mL. The differences in the bacteria distribution could be related to the different activities been carried in the sampling points and the soil type. Beside, the organic constituents of the sampling point can as well influence the population of microorganisms in that soil and might also affect the synthesis of antibiotic production.

In this study, bacteria isolated from soil samples were identified by microbiological protocol like Gram staining (microscopy) and biochemical characterization assays [23,24]. The sum of nine (9) bacteria were isolated from different soil samples including *Corynebacterium diphtheriae*, *Bacillus cereus*, *Escherichia coli*, *Enterobacter aerogene*, *Clostridium* specie, *Bacillus subtilis*, *Pseudomonas* species, *Staphylococcus aureus* and *Klebsiella pneumoniae*. The result obtained from this present study is comparable with the previous studies [26,27,28] who reported the isolation of microbial isolate from soil samples, which were similar to the ones isolated.

Table 1. Total Bacteria count from different sites with various activities of Lapai abattoir

Sites	Activities carried out in the sites	Dilution factor	Number of colonies	Bacteria count (cfu/mL)
North	Water source & washing	10^{-4}	216	2.16×10^6
South	Animal pen	10^{-4}	88	8.8×10^5
East	No activity (vegetation)	10^{-4}	110	1.1×10^6
West	Dung dumping site	10^{-4}	176	1.76×10^6
Centre	Butchering & selling	10^{-4}	104	1.04×10^6

Table 2. Microscopic and biochemical characterization of isolated bacteria

Code name	Grm rxn	Shp	Cat	Coa	Spo	Cit	Ure	Ind	MR	Glu	Lac	Fru	Suc	Identified organism
O1	+	Rod	+	+	-	-	-	-	-	-	-	+	-	<i>Corynebacterium diphtheria</i>
O2	+	Rod	+	-	+	+	-	-	-	+	-	+	+	<i>Bacillus cereus</i>
O3	-	Rod	-	-	-	-	-	+	-	+	+	+	-	<i>Escherichia coli</i>
O4	-	Rod	+	-	-	+	-	-	+	+	+	+	+	<i>Enterobacter aerogenes</i>
O5	+	Rod	-	+	+	+	-	-	-	+	-	+	-	<i>Clostridium specie</i>
O6	+	Rod	+	+	+	+	-	-	-	+	-	+	+	<i>Bacillus subtilis</i>
O7	-	Rod	+	-	-	+	-	-	-	-	-	+	-	<i>Pseudomonas specie</i>
O8	-	Rod	+	-	-	+	-	-	-	-	-	+	-	<i>Pseudomonas specie</i>
O9	+	Cocci	+	+	-	+	-	-	+	-	-	+	+	<i>Staphylococcus aureus</i>
O10	-	Rod	+	-	-	+	-	-	-	-	-	+	-	<i>Pseudomonas specie</i>
O11	-	Rod	+	-	-	+	+	-	-	+	+	+	+	<i>Klebsiella pneumonia</i>
O12	-	Rod	+	-	-	+	-	-	-	-	-	+	-	<i>Pseudomonas specie</i>

Key: - Indicate negative, + Indicate positive, O Indicate organisms 1-12, MR indicate methyl red, Grm rxn indicate gram reaction, Shp indicate shape, Cat indicate catalase, Coa indicate coagulation, Ure indicate urease, Ind indicate indole, Glu indicate glucose, Lac indicate lactose, Fru indicate fructose and Suc indicate sucrose

Table 3. Percentage (%) occurrence of isolated bacteria across the five (5) different sites of Lapai abattoir

Isolated bacteria	Frequency	% Occurrence
<i>Corynebacteriumdiphtheria</i>	1	8.33
<i>Bacillus cereus</i>	1	8.33
<i>Escherichia coli</i>	1	8.33
<i>Enterobacteraerogenes</i>	1	8.33
<i>Clostridium specie</i>	1	8.33
<i>Bacillus subtilis</i>	1	8.33
<i>Pseudomonas specie</i>	4	33.33
<i>Staphylococcus aureus</i>	1	8.33
<i>Klebsiellapneumonia</i>	1	8.33
Total	12	100

Table 4. Zones of inhibition in millimeter (mm) of isolated bacteria against test bacteria

Test isolates	Isolated bacteria (zones of inhibition in (mm))											
	O1	O2	O3	O4	O5	O6	O7	O8	O9	O10	O11	O12
<i>Pseudomonas aeruginosa</i>	-	12	-	-	-	16	-	-	-	-	-	-
<i>Salmonella</i> specie	-	-	-	-	-	-	-	-	-	-	-	-
<i>Klebsiella pneumoniae</i>	-	-	-	-	-	-	-	9	-	-	-	-
<i>Candida albicans</i>	-	-	-	-	-	-	-	-	-	-	-	-

Key:- O1-12 indicates organisms 1-12

This study also agrees in part with the study of Samuel and his co workers regarding the isolated bacteria, but they isolated *Enterococcus faecalis* in addition to those isolated in this present study from the soil [29]. Rajnish [30] also isolated *Pseudomonas fluorescense*, *Streptococcus faecalis* and *Micrococcus luteus* from different soil samples during the course of his research. This variation could be as a result of different activity taking place or geographical location of the sampling sites, as climatological condition might selectively support and favor particular organisms.

From the result obtained in this study, the most prevalent bacterial isolate isolated from the soil was *Pseudomonas* species (33.33%) while *Corynebacterium diphtheriae*, *Bacillus cereus*, *Escherichia coli*, *Enterobacter aerogene*, *Clostridium* specie, *Bacillus subtilis*, *Staphylococcus aureus* and *Klebsiella pneumoniae* 08.33%. This is similar to the result obtained by [22], but argue the literature of Sharga and his other workers that *Bacilli* species were the predominant soil bacteria in the course of study [31].

The present study of primary screening of soil microbes using agar well diffusion method indicated that, three (*B. subtilis*, *B. cereus* and *Pseudomonas* specie) out of 9 bacterial isolates were capable of biosynthesizing antimicrobial metabolites with clear zone of inhibition against some tested microorganisms (*P. aeruginosa* and *K. Pneumoniae*).

Bacillus subtilis show a very good zone of inhibition against *Pseudomonas aeruginosa* which is in agreement with the research of Hassan et al., which reported that *Bacillus* sp. showed high antimicrobial activity against some tested organisms [32] and [33], which stated that *Bacillus subtilis* shows the maximum inhibition zone on certain tested microorganisms [33].

Bacillus cereus was also proved to have antimicrobial activity against *Pseudomonas aeruginosa* in the current finding. The report of previous study also supports our analysis that *Bacillus* species shows higher antimicrobial activity against some pathogenic organisms like *Pseudomonas* sp [33]. It was also observed that *Pseudomonas* species also shows antimicrobial activity with zones of inhibition against *Klebsiella pneumoniae*. *Candida albicans* and *Salmonella* specie resisted all the isolated bacteria from this study. The results of the present study are quite interesting and encouraging because some of the soil bacterial isolates are capable of inhibiting the activity of other microbes which could lead to the development of new antibiotics to treat infectious diseases.

5. CONCLUSION

The results obtained shows that *Bacillus subtilis*, *Bacillus cereus* and *Pseudomonas* specie isolated during the course of this research from soil samples of abattoir in Lapai metropolis have potential of producing antibiotics. The potential of these bacteria to generate antimicrobial substances can be useful for many applications and must be better explored. It is therefore recommended that further analysis by protein electrophoresis and mass spectrometry may help to uncover and the structure of their protein and suggest further investigation regarding characterization using molecular techniques for their identification. The strain improvement by mutagenic agents should be employed to enhance the activity of the strain. Extraction and purification method can be employed for the pure antibiotic production.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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