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Bacterial acrylamidase

Isolation and Screening of Bacteria with Antibiotic-producing Properties From the Soil at the Marmara and New Market Abattoirs, Wukari

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ABSTRACT

Antimicrobials are substances made by organisms that harm other organisms. Rising antibiotic resistance is a global issue, creating a need for alternative treatments. This study screened bacteria with antimicrobial properties from Marmara and New Market abattoirs in Wukari, Nigeria. Twelve soil samples were collected at each location from four sites: slaughtering, animal waste dumping, washing, and point of sale. Samples were taken at three soil depths: 0 cm, 10 cm, and 30 cm, totaling 24 samples. Isolates were screened and preliminarily identified using conventional methods. Eleven (11) bacterial species were isolated, which are *Enterobacter* species (N1, N2, N3), *Pseudomonas* species (N4), *Staphylococcus epidermidis* (N5), *Escherichia coli* (N6), *Staphylococcus aureus* (N7), *Salmonella* species (N8), *Nessieria* species (N9), *Corynebacterium* species (N10), *Proteus* species (N11), *Bacillus* species (N12) and *Klebsiella* species (N13). *Enterobacter* species was identified as the most frequently isolated organism. These isolates were further screened against some known human pathogens; *Escherichia coli, Pseudomonas aeruginosa*, *Staphylococcus aureus, Klebsiella pneumoniae* and *Salmonella* species. The result showed that one of the *Enterobacter* species (N1) has activity on all the pathogens, the other *Enterobacter* species (N2) has activity against four except *Klebsiella pneumoniae* and the last *Enterobacter* species (N3) has activity also against four pathogens except *Salmonella* species while *Pseudomonas* species have activity against three of the pathogens except *Klebsiella pneumonia* and *Escherichia coli.* Lastly, *Staphylococcus epidermidis* has activity on two pathogens and no activity on *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Escherichia coli*. This study showed that some soil microorganisms could be an interesting source of antimicrobial substances against some human pathogens. Pharmaceutical industries can explore this ability during antimicrobial production and synthesis.

INTRODUCTION

Drug-resistant strains are becoming more prevalent, mainly acquired multidrug resistance strains, a major global health risk spreading quickly [1]. This is due to the abuse and overprescription of antibiotics, which has hampered our capacity to provide empirical patient care [2,3]. Many bacteria have evolved resistance to these widely used antibiotics through processes like decreased cell wall permeability, the efflux pump, and beta-lactamases hydrolysis of the beta-lactam ring. The most significant method by which pathogenic bacteria develop resistance to the current generation of non-beta lactam antibiotics is the synthesis of metallobetalactamases [4]. These have made the urgent search for and development of novel medications necessary in modern times to tackle infectious diseases and reduce the spread of antibiotic resistance [5]. Antibiotics, which are secondary metabolites synthesized by microorganisms possessing antibacterial properties, have been employed as a chemotherapeutic agent against pathogens for several years. The utilization of antibiotics, in conjunction with adequate hygiene and vaccination practices, has markedly decreased mortality rates stemming from predominantly lethal infections [6]. The identification and separation of antibiotics from microorganisms, in lieu of chemical sources, has resulted in the unearthing of

innumerable special antibiotics [7]. Antibiotics play a critical role in averting and treating microbial infections in diverse sectors of human advancement [8]. Due to its heterogeneous nature, the soil harbors an extensive and varied range of microorganisms [9]. The diverse biotic and abiotic soil conditions lead to the adaptation and formulation of survival and reproductive strategies among microbial residents. One of the most efficacious adaptation mechanisms is the synthesis of antimicrobial agents, as asserted by Davies [10]. Pathogenic microorganisms have long been exposed to several natural and synthetic antibiotics and in the process, have evolved multiple defense mechanisms against them. The pervasive employment of antibiotics and disinfectants in medical practices, agricultural undertakings, and aquaculture, and their consequent discharge into the surrounding milieu, presents a profound quandary of multidrug resistance [11,12].

Resistance establishment can occur rapidly when antimicrobials are targeted at a single mechanism or enzyme responsible for cell death. In contrast, antibiotics that act against multiple targets or mechanisms to kill microbes develop resistance more slowly, as noted by Demain and Sanchez [12]. In light of this, the formation of fresh and effective antibiotics to combat emerging resistant strains is in dire need. One of the most powerful choices for discovering new chemotherapeutic agents is through natural metabolites from microorganisms. Soils from different ecosystems and biogeographic regions can be analyzed to discover new antimicrobial-producing strains with novel mechanisms of action and secondary metabolites for disease-resistant microorganisms [13]. This research work was undertaken with an effort to isolate, identify and further characterize antibiotic-producing bacteria and also to assess the inhibitory properties of the isolated bacteria from different soil samples at Marmara and New market abattoir in Wukari, Taraba State, Nigeria, against some human pathogenic microbes.

MATERIALS AND METHODS

Study area

The study area was Wukari, Local Government Area in Taraba State, Nigeria. It's headquarter is in Wukari town, located along 200 Katsina-ala road at latitude $7^0$52' 13.9" north and longitude $9^0$46' 39.2" east. It has an area of 4,308 km^2 and a population of 241,546 at the 2006 census. The soil texture is generally classified as loamy sand, sandy loam, sandy clay and sand. The rainy period spans 8.5 months, from March 4 to November 19, with a minimum 31-day rainfall of 0.5 inches. The climate is tropical savanna with an average annual temperature of 33 ºC. Due to the increase in population as a result of the establishment of Federal University Wukari and Kwararafa University Wukari, many more slaughtering areas (abattoir) were formed [14].

Sample collection

Two (2) abattoirs, namely, Marmara and New Market abattoirs in Wukari were selected and used for this study. At each abattoir location, soil samples were respectively collected from four (4) separate (slaughtering, animal waste dumping, washing and point of sale) sites and at three (3) levels of soil depth (0cm - top soil, 10 cm and 30 cm) and transferred into well-labeled dry and clean polythene bags (24 soil samples). These bags were tightly sealed and immediately transported to the microbiology laboratory located at Federal University Wukari, Taraba State, Nigeria where the entire research work was carried out.

Sample preparation and isolation of bacteria

Twenty-four (24) clean test tubes, each containing 9 mL of distilled water were sterilized in an autoclave at 121 ºC for 15 min. Thereafter, one (1) gram of soil sample from each site was accurately weighed, suspended in 9 mL of distilled water and vortexed for 2-3 min to obtain a homogenous mixture. From the stock culture, a volume of 1 mL was aseptically transferred to the next tube and serially diluted accordingly [9] on a ten-fold dilution technique. Subsequently, a volume of 1 mL of soil suspension from each 10^{-6} serially diluted tube was transferred into a nutrient agar plate and evenly spread with a sterile L-shaped glass rod. This procedure was repeated using Eosin Methylene Blue agar and Mueller Hinton agar plates. All the plates were finally aerobically incubated at 37 ºC for 24 h [15].

Identification of the bacteria and bacterial isolates

After an incubation period, the plates underwent a thorough observation process. A solitary loopful of distinguishable colonies from the plates was cautiously selected and subjected to additional sub-culturing on newly prepared nutrient agar and successively streaked to ensure purity [16]. This process was carried out for 24 h at a temperature of 37 ºC in order to procure pure cultures that could be utilized for subsequent microbiological and biochemical analyses. Pure single colonies were noticed after 24 h of incubation at 37 ºC from the procured pure culture plates. Bacterial isolates were identified based on their colony morphology microscopic characters and further confirmed through biochemical tests [17,18,19].

Antimicrobial assay

Standardized (test) organisms (*Staphylococcus aureus*, *Klebsiella pneumoniae, Escherichia coli, Pseudomonas aeruginosa* and *Salmonella* species) that were sourced from the National Veterinary Research Institute, Vom, Plateau State, were used to determine the antibiotic-producing abilities of the isolated (experimental) soil bacteria. Each bacterial isolate was streaked along the diameter of an agar plate which was incubated at 37 ºC for 24 h. Each test organism was then standardized to 0.5 McFarland standard (1.5 x 10^8 cfu/mL at 560 nm) and streaked perpendicular to the isolate. The plates were further incubated at 37 ºC for 24 h. The antimicrobial activity was estimated from the zone of inhibition of test organisms [20].

RESULTS

Table 1 shows the different activities carried out at the sites, soil depth, dilution factors, number of colonies and bacteria colony counts. The microbial load presence at the different studied sites of abattoirs in Marmara and New Market varied from point to point. Bacteria load was recorded high at the slaughtering sites too numerous to count (TNTC) and the lowest number at the dumping sites 7.8×10^6. **Table 2** shows a total of thirteen (13) bacterial isolates identified in this study and their respective shapes during microscopy and action on different biochemical tests. The isolates include *Enterobacter* species(N1), *Enterobacter* species(N2), *Enterobacter* species(N3), *Pseudomonas* species (N4), *Staphylococcus epidermidis* (N5), *Escherichia coli* (N6), *Staphylococcus aureus* (N7), *Salmonella* species (N8), *Nessieria* species(N9), *Corynebacterium* species (N10), *Proteus* species (N11), *Bacillus* species (N12) and *Klebsiella* species (N13).

Table 3 shows the percentage (%) occurrence of isolated antibiotic-producing bacteria in four (4) different sites (slaughtering, animal waste dumping, washing, and point of sale) of Marmara and New Market abattoirs soil in Wukari. Enterobacter species was observed to be the most frequently isolated bacteria (60%) while the rest of the isolates were 20%.
Table 4 shows the zone of inhibition in millimeter (mm) of the thirteen isolated bacteria: *Enterobacter* species (N1), *Enterobacter* species (N2), *Enterobacter* species (N3),

Pseudomonas species (N4), *Staphylococcus epidermidis* (N5), *Escherichia coli* (N6), *Staphylococcus aureus* (N7), *Salmonella* species (N8), *Nessieria* species (N9), *Corynebacterium* species (N10), *Proteus* species (N11), *Bacillus* species (N12) and *Klebsiella* species (N13)] against the five test bacteria (*Escherichia coli, Pseudomonas aeruginosa*, *Staphylococcus aureus, Klebsiella pneumoniae* and *Salmonella* species). The zone of inhibition of *Enterobacter* species (N1), *Enterobacter* species (N2), *Enterobacter* species (N3), *Pseudomonas* species (N4) and *Staphylococcus epidermidis* (N5) were observed against the test bacteria while the rest of the isolates had no zone of inhibition.

Table 1. Total bacteria count from Marmara and New Market abattoir soil. (Dilution factor is 10^6).

Serial Number	Activities carried out at the sites	Soil depth depth (cm)	Number of colonies	Bacteria colony counts (cfu/mL)
1	PSS	10	86	1.72×10^7
2	WSS	0	376	TNTC
3	WSS	0	146	2.92×10^7
4	SSS	10	182	3.64×10^7
5	SSS	30	162	3.24×10^7
6	SSS	0	480	TNTC
7	SSS	0	47	9.4×10^6
8	DSS	0	39	7.8×10^6
9	WSS	30	275	5.5×10^7
10	WSS	30	40	8×10^6
11	DSS	30	350	TNTC
12	WSS	10	35	7×10^7
13	WSS	30	33	6.6×10^7

KEY: PSS = point of sale soil, WSS = washing site soil, SSS = slaughtering site soil, DSS = dumping site soil, TNTC = too numerous to count

Table 2. Characteristics of screened microorganisms isolated from MA and NMA soil sample.

Samples Number	Gram reaction	Biochemical reaction Catalase	Coagulase	Microorganism
N1	- rod	-	-	*Enterobacter* spp
N2	- clustered rod	-	-	*Enterobacter* spp
N3	- rod	-	-	*Enterobacter* spp
N4	- coccobacillus rod	+	-	*Pseudomonas* spp
N5	+ cocci	+	-	*Staphylococcus epidermidis*
N6	- clustered rods	-	-	*Escherichia coli*
N7	+ cocci (in clustered)	+	+	*Staphylococcus* spp
N8	- rod	-	-	*Salmonella* spp
N9	- cocci	+	-	*Neisseria* spp
N10	+ rod	+	-	*Corynebacterium* spp
N11	- clustered rods	-	-	*Proteus* spp.
N12	+ rod	+	-	*Bacillus* spp
N13	- rod	-	-	*Klebsiella* spp

MA = Marmara Abattoir, NMA = New Market Abattoir

Table 3. Percentage (%) occurrence of isolated antibiotic-producing bacteria in four (4) different sites of Marmara and New Market abattoir soils in Wukari.

Isolated bacteria	Frequency	% Occurrence
Enterobacter specie	3	60
Pseudomonas specie	1	20
Staphylococcus epidermidis	1	20
Total	5	100

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

Test isolates	Isolated bacteria (zones of inhibition in (mm)) N1	N2	N3	N4	N5	N6	N7	N8	N9	N10	N11	N12	N13
Escherichia coli	22	14	16	-	-	-	-	-	-	-	-	-	-
Pseudomonas aeruginosa	20	21	18	30	-	-	-	-	-	-	-	-	-
Staphylococcus aureus	21	19	20	29	18	-	-	-	-	-	-	-	-
Klebsiella pneumoniae	23	-	17	-	-	-	-	-	-	-	-	-	-
Salmonella specie	25	24	-	29	25	-	-	-	-	-	-	-	-

KEY: N1-*Enterobacter* species, N2-*Enterobacter* species, N3-*Enterobacter* species, N4-*Pseudomonas* species, N5-*Staphylococcus epidermidis*, N6-*Escherichia coli*, N7-*Staphyloccus aureus*, N8-*Salmonella* species, N9-*Neisseria* species, N10-*Corynebacterium* species, N11-*Proteus* species, N12-*Bacillus* species, N13-*Klebsiella* species.

DISCUSSION

The soil harbors a diverse array of microflora, which presents opportunities for the discovery of novel species and strains with the potential to produce innovative antimicrobial agents. The present research endeavors to elucidate the bacteriological profile of soil samples obtained from two distinct abattoirs. The scope of this investigation encompasses the isolation of previously unreported bacterial strains from soil specimens, which were then subjected to rigorous screening protocols to assess their antimicrobial efficacy against various pathogenic microorganisms.

Notably, the sampling locations exhibited marked discrepancies in bacterial density; the highest bacterial load was recorded at the slaughtering site TNTC= too numerous to count which may be attributed to the rich blood meal that serves as a good source of nutrients for the bacteria, while the lowest bacterial count was at the dumping site $7.8x10^6$ which may be as a result of the waste, depletion of nutrient and toxic environment for the bacteria couple with the fact that range of factors, including variations in the nature of activities conducted at each site, as well as differences in soil type and organic constituents. This study employed established microbiological techniques such as Gram staining (microscopy) and biochemical characterization assays to identify the bacterial isolates accurately.

The isolation of eleven (11) bacterial strains from two (2) distinct soil samples, comprising *Enterobacter* species (N1, N2, N3), *Pseudomonas* species (N4), *Staphylococcus epidermidis* (N5), *Escherichia coli* (N6), *Staphylococcus aureus* (N7), *Salmonella* species (N8), *Neisseria* species (N9), *Corynebacterium* species (N10), *Proteus* species (N11), *Bacillus* species (N12) and *Klebsiella* species (N13), was conducted. The findings from this current investigation are akin to those of prior studies by [9], which reported the isolation of microbial strains from soil samples comparable to the ones isolated in this study. Based on the results of this current study, the most common bacterial strain identified in the soil from both abattoirs was *Enterobacter* species, which contradicts the work of [21], which identified *Bacilli* species, and [9], which identified *Pseudomonas* species. This variation could be attributed to the sampling sites' divergent activities or geographic regions, as climatological conditions may selectively support and favor specific organisms.

The current study of primary screening of soil microbes using the perpendicular method revealed that five *Enterobacter* species (N1), *Enterobacter* species(N2), *Enterobacter* species (N3), *Pseudomonas* species(N4), and *Staphylococcus epidermidis* (N5)} of the thirteen (13) bacterial isolates were capable of producing antibiotic properties with a clear zone of inhibition against some five tested pathogens (*Escherichia coli, Salmonella* species, *Pseudomonas aeruginosa, Klebsiella pneumoniae*, and *Staphylococcus aureus*).

The *Enterobacter* species (N1) from Marmara abattoir point of sale site shows a perfect zone of inhibition against the five (5) tested pathogens and *Enterobacter* species (N2) isolated from New Market washing site show complete activities on four (4) pathogens and resistance to (*Klebsiella* species). In contrast, the second *Enterobacter* species (N3) isolated from New Market washing site had activities on four (4) except (*Salmonella* species) all these agreed with the work of Chernin *et al*. [22], which reported that *Enterobacter* species showed high antimicrobial activity against some tested pathogens and the antibiotic production of *Enterobacter* species might be the reason

why other organisms were almost non-existence. Also, *Pseudomonas* species isolated from New Market slaughtering site soil show activity on three pathogens and resistance to (*Escherichia coli* and *Klebsiella pneumoniae*), which the previous study reported by Uwalaka *et al*., supports this analysis [9]. Furthermore, lastly, *Staphylococcus epidermidis* (N5) shows activities on two (2) pathogens and resistance to three (3) pathogens (*Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*). The result of this research is intriguing and encouraging because some soil bacterial isolates can inhibit the activity of other microorganisms, which could lead to the development of new antibiotics to treat infectious diseases.

CONCLUSION

From this study, it can be seen that there is a great deal of microbial activity in the abattoir soils and that there may be a potential for the production of antibiotics from these soils. The bacterial load of the soil samples taken from Marmara and New Market abattoirs differed significantly, with the highest count being seen at the slaughtering sites (TNTC). This is because the sites are nutrient-rich, while the dumping sites had the lowest count of 7. 8×10^6 CFU/mL. Thirteen bacterial isolates, including Enterobacter species, Pseudomonas species and *Staphylococcus epidermidis*, were identified through microbiological techniques. Out of these, Enterobacter species was the most frequently isolated bacteria (60%) and had antimicrobial potential. For instance, the *Enterobacter* species isolates (N1, N2, N3) showed ability to inhibit the growth of multiple pathogens. Other isolates, such as *Pseudomonas* species and *Staphylococcus epidermidis,* also showed activity on some pathogens but to a certain degree. The results of this study are in accordance with some previous research works, but at the same time, they are in contrast with some of the previous findings indicating the effect of geography and environment. In general, the outcomes of this research demonstrate the possibility of soil microorganisms in abattoirs as a source of new antibiotics to combat the increasing problem of antimicrobial resistance. These preliminary results open up new possibilities for identifying and creating new antimicrobial agents based on microbial products.

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