Golden Sun-Rise
International Journal of Multidisciplinary on Science and Management
ISSN: 3048-5037/ Volume 2 Issue 2 Apr-Jun 2025 / Page No: 150-158
Paper Id: IJMSM-V2I2P114/ Doi:10.71141/30485037/V2I2P114

Research Article

Molecular Characterization of Bacteria Isolated from Soil Sample Collected from Oyscatech Pig House

Bamigboye Oluwabunmi Florence[1], Amusat Afusat Ibiwumi[1], Bakare Bisola Adebola[1], Aribisala, Lukman Aderemi[1], A.O. Olalekan[1], David Timilehin Dorcas[1], Ntagbu Folasade Gift[2], Ogunwole Michael Ogunmola[3]

[1]Department of Science Laboratory Technology, Oyo State College of Agriculture and Technology, Igboora, Oyo state, Nigeria.
[2]Department of Animal Production (Microbiology Unit) Federal College of Wild Life Management, New Busa, Niger State, Nigeria.
[3]Department of Pure and Applied Biology, Ladoke Akintola University of Technology, Ogbomoso, Oyo State, Nigeria.

Received: 10 May 2025 Revised: 15 May 2025 Accepted: 20 May 2025 Published: 31 May 2025

Abstract - *This investigation describes the isolation and molecular characterization of bacteria isolated from soil samples collected from Pig House in Oyo State College of Agriculture and Technology, Igboora. Isolation from soil samples was carried out and the isolated organisms were characterized using biochemical tests and molecular characterization. Two isolates were Gram-negative while sixteen were Gram-positive they are: Staphylococcus aureus 38.8 %, Staphylococcus epidermidis 16.7%, Streptococcus spp 11.1 %, Neisseria spp 11.1 %, Enterococcus spp 5.6 %, Micrococcus luteus, 5.6% Salmonella enterica 5.6% and Escherichia coli 5.6%. DNA extraction was carried out using Zyms quick DNA Bacteria. The Polymerase Chain Reaction (PCR) was used Phylogenetically to predict the likely bacteria DNA of the selected organism and the relationship between them when compare with the GenBank and the isolates were identified as Escherichia coli and Salmonella enterica. This study provided information on some bacteria harbored by pig house soil which could be a medium of transmission of diseases to animals, handlers and the community. Therefore, proper sanitary measures should be adopted in the pig house to avoid transmission of diseases from the pig to the human through handling and when consume as meat.*

Keywords - *DNA, molecular characterization, Pig house, isolation, Phylogenetic, Polymerase Chain Reaction.*

I. INTRODUCTION

Pigs are stout-bodies with legged, omnivorous mammals, with thick skin which are usually sparsely coated with shout bristles. Their hooves have two functional and two nonfunctional digits. pigs are mammal belonging to the order Artiodactyla, family Suidae and the shelter of a pig is called a sty. Mishandling of pig farm waste and animal droppings may impact negatively on the physical environment, especially polluting the soil with bacteriological pathogens. The pollution may consequentially cause serious waterborne and airborne diseases as a result of ingestion, direct contact or inhalation of contaminated aerosols (Ramírez et al., 2005).

Potential sources of bacterial pollution in pig farms include feedlot pastures, treatment lagoons, manure storage, and also land application fields (Hong et al., 2013). Pathogens can be transported in soil receiving waste through movement with infiltrating water, and surface run-off water and with the movement of sediments and waste particles (Jamieson et al., 2002). Pig farming produces emissions of biological (microbes), mechanical (dusts) and chemical (gases) contaminants. Microbial contamination of animal environment constitutes one of the most profound health and life hazards to animals during the raising period. It is associated with confinement of high numbers of animals per unit area that contributes to considerable pollution of air and bedding material in pig facilities (Buczyńska and Szadkowska-Stańczyk, 2010). Studies determined the presence of numerous microorganisms in air of the swine facilities, the most frequently isolated bacteria included Escherichia coli,

Staphylococcus xylosus, Micrococcus luteus, Streptococcus uberis, Leuconostoc lactis and Shigella spp., Enterococcus faecium and Enterococcus faecalis (Beata, et al., 2015).

A major source of animal protein and contributor to human health, is scaling up and intensifying. The Food and Agricultural Organization (FAO, 2022) reported that the average global swine inventory was 1.36 billion heads from 2016 to 2020, and the numbers of pigs in stock may increase in the future. Along with the production intensification and scale-up, air pollution in swine industry has raised great concern, especially under the context of One Health that emphasizes the relatedness of human, animal, and environmental health in disease control and prevention strategies (One Health Commission, 2018). Air exhausted from swine farms contains vast odorous chemicals and particulate matter (PM). Suspended airborne PM with diameters ranging from 0.001 to 100 μm could form aerosols (Georgakopoulos et al., 2009). The airborne resistance genes could travel up to 10 km through wind, increasing the possibility of infection for humans and animal (Bai, et al., 2022, Wang, et al., 2021). Gladding et al., (2020) reported that the concentrations of airborne culturable bacterial, fungi, Staphylococcus aureus, and endotoxin in swine farms were much higher than the background concentrations. They even maintained a high level up to 250 m away from the farms downwind. Inhalation is a major and common transmission route of airborne microorganisms from farm environments to human and swine bodies, causing respiratory infection and impaired lung function (Kraemer, et al., 2018, Tang, et al., 2021).

Bacteria habitually identified in a pig house also include E. coli, which is a component of the gastrointestinal tract flora and often trigger conditions associated with diarrhoea (Kiers et al., 2007; Weiner et al., 2004). It is also thought that better knowledge of the factors affecting the survival of pathogenic strains of E. coli in the soil facilitates their more efficient control and prevents the transfer of these microbes to food products (Habteselassie et al., 2008, (Trawińska, et al., 2015). Bacterial development and survival in soil is favoured by high temperature and moisture (Boes et al., 2005). Substantial bacterial contamination also pertains to the area surrounding largescale livestock farms.

Tymczyna et al., (1999) studying groundwater samples taken from the surroundings of a swine farm showed the presence of E. coli, fecal streptococci, Clostridium perfringens and Pseudomonas spp., whereas Corynebacterium pseudotuberculosis, E. coli, Clostridium perfringens, faecal streptococci, Bacillus subtilis and Proteus spp. were determined in soil samples. It is noteworthy to highlight a vital role of the environmental reservoir in the incidence of Salmonella-induced infections in pigs (Hoelzer et al., 2011). Microbial contamination of animal faeces and natural environment, especially the presence of pathogenic bacteria, may pose human and animal health hazard (Trawińska, et al., 2015). Therefore, this study aims at characterizing the bacteria isolated from the soil sample collected from pig house of Oyo State College of Agriculture and Technology, Igboora through phenotypic, biochemical and phylogenetic analysis.

II. MATERIALS AND METHODS

A. Collection of Soil Samples

Two soil sample (digged and surface) was collected from Oyo state College of Agriculture and Technology (Oyscatech) pig house in Igboora. The samples were taken with the help of sterile spatula, into a sterile Ziplock nylon and then taken to the Research laboratory under a hygienic condition for microbiological Analysis.

B. Isolation of Bacteria from Soil

The serial dilution was prepared by adding 10 g of the sample to 90 mls of distilled water and mixed well for 15 min and vortexed. Tenfold serial dilution was carried out and spread plate method was used to isolate bacteria from dillution 10-4, 10-6 and 10-8 into a freshly prepared Nutrient Agar plates, the plates were incubated at 37 0C for 24 hrs. The colony that appears on the plates were counted, recorded and considered as one colony forming per unit (cfu).

C. Identification and Characterization of Bacteria

Pure culture of the isolated organisms was subjected to Gram staining to check the morphology of the organisms isolated. The isolates were then characterized biochemically and two of the organisms isolated were subjected to molecular characterization using16SrRNA Gene Amplification.

D. Molecular characterization

a. 16SrRNA Gene Amplification of the Bacterial Isolate

The PCR mix is made up of 12.5µL of Taq 2X Master Mix from New England Biolabs (M0270); 1µL each of 10µM forward (27F: AGAGTTTGATCMTGGCTCAG) and reverse (1525R: AAGGAGGTGWTCCARCCGCA) primer; 2µL of DNA template and then made up with 8.5µL Nuclease free water.

E. Phylogenetic Analysis

Sequence was edited and trimmed on MEGA X and was blasted for identification of species on National Centre for Biotechnology Information (NCBI) database. The sequence was compared with other ITS1-2 gene sequences in GenBank and aligned using Clustal W. Best BLAST hits were used for the construction of neighbor-joining phylogenetic tree. Evolutionary analysis was performed on MEGA X software version 10.0.1 (Kumar et al., 2018).

F. Cycling Conditions for the Amplification of the 16SrRNA Gene

Initial denaturation was done at 94˚C for 5mins, followed by 36 cycles of denaturation at 94˚C for 30sec, annealing at 56˚C for 30secs and elongation at 72˚C for 45 sec. The final elongation step was done at 72˚C for 7 minutes at 10 ˚C.

G. Internal Transcribed Spacer (ITS) Gene Amplification of the Bacteria Isolated

The PCR mix was made up of 12.5µL of Taq 2X Master Mix from New England Biolabs (M0270); 1µL each of 10µM forward (ITS 1: TCC GTA GGT GAA CCT GCG G) and reverse primer (ITS4 TCCTCCGCTTATTGATATGS); 2µL of DNA template and then made up with 8.5µL Nuclease free water and DNA extraction was carried out using ZR bacterial DNA miniprep (manufactured by zymo research). Electrophoresis for DNA and PCR was also carried out as well as Loading of Samples and Running on Agarose Gel.

H. Sequencing

The amplified fragments were sequenced using a Genetic Analyzer 3130 x 1 sequencer from Applied Biosystems using manufacturers' manual while the sequencing kit used was that of Big Dye terminator v3.1 cycle sequencing kit. Bio- Edit software and MEGA X were used for all genetic analysis.

III. RESULTS AND DISCUSSION

A. Result

Result from table 1, reveals that pig digged soil sample have the highest microbial load than the surface soil samples collected. Dilution 10-4 of pig digged soil have the highest viable count of 54 while, 10-8 have the lowest count of 10 in 10-8. Result from biochemical test in table 2 shows the identification of 18 organisms namely, Streptococcus spp 11.1%, Staphylococcus aureus 38.8%, Enterococcus spp 5.6%, Staphylococcus epidermidis 16.7%, Neisseria spp 5.6%, Escherichia coli 5.6%, Salmonella enterica 5.6%, Micrococcus aureus 5.6%, which include Gram-positive and Gram-negative bacteria. Figure 1 represent the percentage of occurrence of each of the organism isolated from the soil samples collected from the Pig house. Figure 2 shows the Molecular weight of the DNA extracted from the bacteria isolates subjected to molecular characterization with their accession number and codon where PS/S10-4 has 80.64 % pairwise similarity with Salmonella enterica strain Inspire 69 which has NCBI accession number JQ315905 and PD/S10-6 has 99.64 % pairwise similarity with Escherichia coli strain EGE 4903307-101which has NCBI accession number KY655124.Figure 3 shows the 1.5% Agarose gel electrophoresis of the 16SrRNA gene amplification of the bacteria isolates at 1500bp. Figure 4 represent the phylogenetic tree of the organisms isolated which shows the Neighbor-joining phylogenetic dendrogram based on a comparison of the 16S rRNA gene sequences of the Gram-negative representative isolates and some of their closest phylogenetic taxa.

Table 1. Total Viable Count of the organisms Isolated from Pig House Soil

Isolate	Dillution factor (cfu/g)		
	10^{-4}	10^{-6}	10^{-8}
PD/S	55	25	10
PS/S	35	22	10

Key: PD/S -Pig Dig Soil, PS/S- Pig Surface Soil

Table 2. Biochemical Characterization of Microorganisms Isolated from Soil Sample Collected from Pig House

ISOLATE	Gram Reaction	Catalase Test	Glucose fermentation	Mannitol fermentation	Starch hydrolysis	Fructose fermentation	Lactose Fermentations	Spore Staining	Oxidase	VP Test	Swollen Cell	Growth at 65 °C NaCl	Yellow Pigment	Novobiocin sensitivity	Probable Organismsk
PS/S 101^{-8}	+C	-	+G)	-	-	+	+	-	-	-	-	-	-	-	*Streptococcus* spp
PS/S 103^{-8}	+C	+	+(G)	+(G)	-	+	+	-	-	-	-	-	-	-	*Staphylococcus aureus*
PD/S103^{-6}	+C	+	+(G)	+(G)	-	+	+	-	-	-	-	-	-	-	*Staphylococcus aureus*
PD/S102^{-4}	+C	+	+(G)	-	-	+	+	-	-	-	-	-	-	-	*Staphylococcus epidermidis*
PD/S102^{-6}	+C	+	+(G)	+(G)	-	+	+	-	-	-	-	-	-	-	*Staphylococcus aureus*
PD/S101^{-6}	+C	+	+(G)	-	-	+	+	-	-	-	-	-	-	-	*Staphylococcus epidermidis*
PD/S103^{-4}	+C	+	+(G)	+(G)	-	+	+	-	-	-	-	-	-	-	*Staphylococcus aureus*
PD/S103^{-8}	+C	+	-	-	-	+	+	-	-	-	-	-	+	-	*Micrococcus luteus*
PS/S103^{-4}	+C	+	+(G)	+	-	+	+	-	-	-	-	-	-	-	*Staphylococcus aureus*
PS/S101^{-4}	+C	+	+(G)	-	-	+	+	-	-	-	-	-	-	-	*Staphylococcus epidermidis*
PD/S102^{-8}	+C	+	+(G)	+(G)	-	+	+	-	-	-	-	-	-	-	*Staphylococcus aureus*
PS/S102^{-4}	-R	-	+(G)	+(G)	-	+	-	-	-	-	-	-	-	-	*Salmonella enterica*
PD/S 101^{-4}	-C	+	+(G)	+(G)	-	+	+	-	-	-	-	-	-	-	*Neisseria* spp
PD/S101^{-8}	-C	+	+(G)	+(G)	-	+	+	-	-	-	-	-	-	-	*Neisseria* spp
PS/S102^{-8}	-C	-	+(G)	-(G)	-	+	+	-	-	-	-	-	-	-	*Enterococcus* spp
PS/S102^{-6}	+C	+	+(G)	+(G)	-	+	+	-	-	-	-	-	-	-	*Staphylococcus aureus*
PS/S101^{-6}	-R	-	-	-	+	+	+	-	-	-	-	-	-	-	*Escherichia coli*
PS/S103^{-6}	+C	-	-	-	+	+	+	-	-	-	+	-	-	-	*Streptococcus* spp

Keys: PD/S: Pig Digged Soil, PS/S: Pig Surface Soil

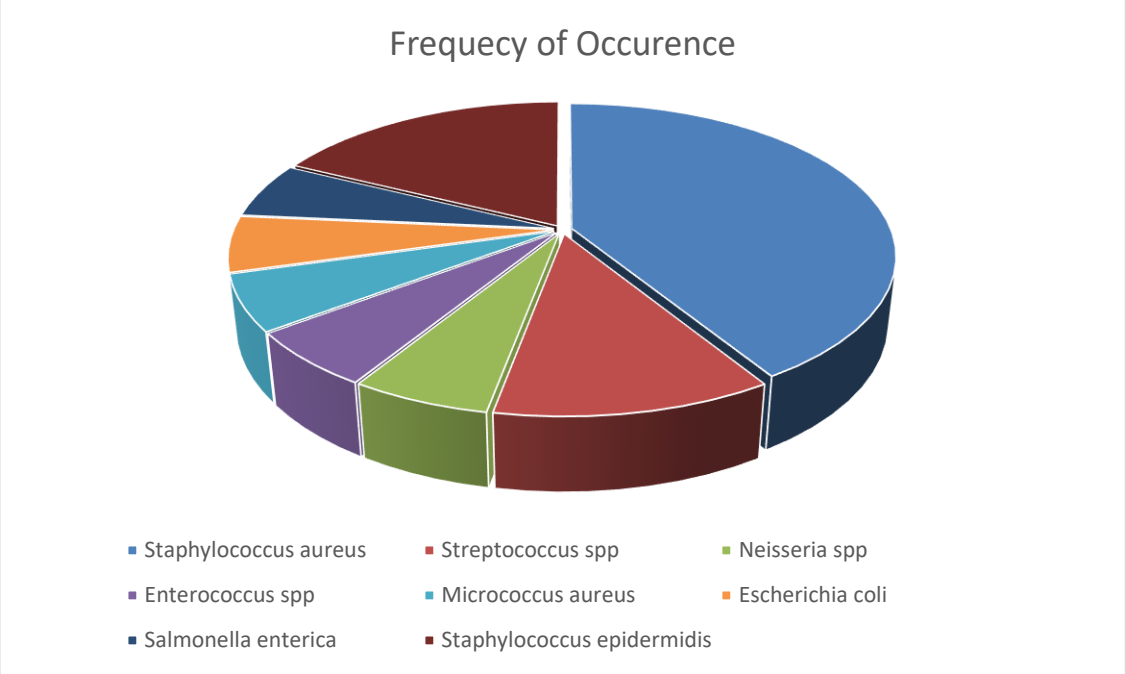

Figure 1. Percentage Occurrence of the Organisms Isolated from Soil Samples Collected from Pig House

Figure 2. Gel image of high molecular weight DNA extracted from the bacteria isolates. Lane B1 = PS/S² 10^{-4}, Lane B2 = PD/S2 10^{-6}

>PS/S10^{-4} has 80.64 % pairwise similarity with Salmonella enterica strain Inspire 69 which has NCBI accession number JQ315905. The e value is 2.00E-45

TGGCCCGGTGTCCGGAATTAATGGGCGTAAGGCACGCAGCTGCTTATTCAGTCCCAGTTAAATCCCCGGTCTAACCTGGG
ATTGCATTCGAACTGGTGGCTTGAGCTTGTCATTGTGGTAATTTGCAGTGTGCGGTGAAATGCTAAAGATTTGGAGGAAT
CCCCGGGCCAACGCGGTCCTCTGGACCAGACCGATTCTGAGGCGCGAAAGCGTGGGGAGCAAAAAGGATTAGATACCCTG
GTAGTCATGCCGTAAACCCTGTCTTTTGAGGTTGGGCATTGAGGCTTGCTTCCCTGGCTACGCGTCAATC

>PD/S10^{-6} has 99.64 % pairwise similarity with Escherichia coli strain EGE 4903307-101 which has NCBI accession number KY655124. The e value is 0

TGGGTGGGGGGGGGGGGGGGGGCGTTACGGATTCTGGGGGTAACGCACGCAGGCGGTTTCTTAGTCTGAGGGAAGCCCCC
GGCTCAACCTGGGAATGCATTGGAACTGGCTGGCTTGAGTCTTGACCAGGGGAGTAGAATTCCATGTGTAGCGGTGAAAT
GCGTAGAGATCTGGAGGAATACCGGTGGCGAACGCGGCCCCCTGGGCTGAAACTGACGCTCAGGCGCGAAAGCGTGGGGA
GCAAACAGGGTTAGATACCCTGGTAGTCCACGCCGTAAACGATGTCGGCTTAGAGGTTGAGGCCTTGCACCCTGGCTTCC
TGCGCCAACGGGTTTAATCGACCGCCCGGGGAATAAGGACGCCAGGGTTAAAACTAAATGAATTGAAGGGTGCCCCCCCA
AGCCGTGGAATAAGGGGTTTTATTTCCTGCTGGGCCGGAACCTTAACTTCTTTTGACTTTCTTTAAGCTTTCAAAAACCC
AAGGTGGCCTTAGGAACCTAAGACAGGTGCTGGCATGGCTGGCGTCAGCTCGCGTTTGTGAAATGGTGGGTTTAAGGCCG
CGCAACAACAGGAGGCCCTTAATCTTTGT

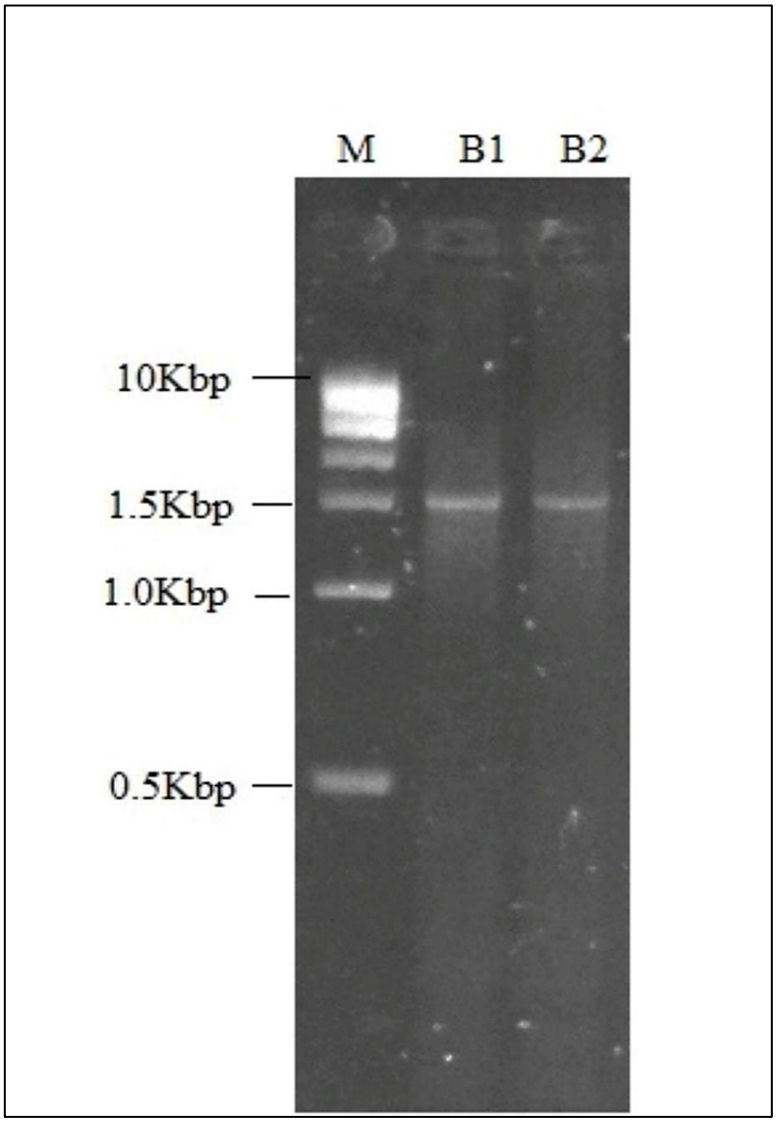

Figure 3. 1.5% Agarose Gel Electrophoresis of the 16SrRNA Gene Amplification of the Bacteria Isolates at 1500bp. M is a 1Kbp DNA ladder. Lane B1 = P/D² 10^{-6}, Lane B1 = P/S2 10^{-4}

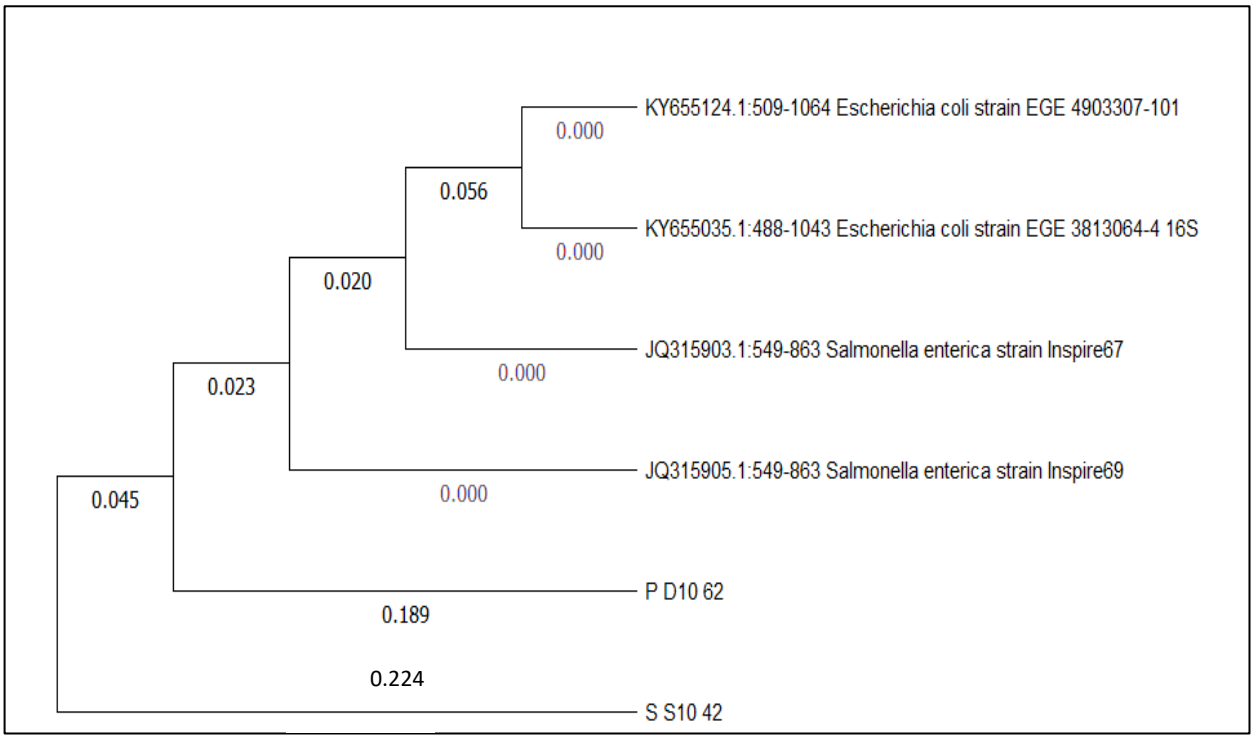

Figure 4. The Neighbor-Joining Phylogenetic Dendrogram Based on a Comparison of the 16S rRNA Gene Sequences of the Gram-Negative Representative Isolates and Some of their Closest Phylogenetic Taxa

B. Discussion

This work reveals the microorganisms associated with the soil samples collected from Oyo State College of Agriculture and Technology, Igboora Pig house where the highest viable count was discovered from the digged soil sample collected with total number of 55×10-4 this work is in line with the work done by Trawińska et al., (2015) in his work titled, Effect of Pig Farm on Microbial Contamination of Soil where the greatest bacterial load was found in the manure samples collected at 1/2 length of the pig facility KIII.

Twenty-one organisms were characterized biochemically and these are; Streptococcus spp 11.1%, Staphylococcus aureus 38.8%, Enterococcus spp 5.6%, Staphylococcus epidermidis 16.7%, Neisseria spp 5.6%, Escherichia coli 5.6%, Salmonella enterica 5.6%, Micrococcus aureus 5.6%, which include Gram-positive and Gram-negative bacteria. This work is in line with the work done by Jurek, et al., 2006 and Kluczeks, 2002. E. coli found in this work is in line with the work done by Trawińska et al., (2015) where E. coli was determined in the soil samples collected 15 m off the building wall (GII). Hoelzer et al., (2011) and Nowak et al., (2007) also isolate Salmonella as an induced infections in pigs house which is also in line with this work. Streptococcus spp and Enterococcus spp isolated from pig house soil sample is also in line with the work done by Sanz et al., 2018.

The PS/S10-4 characterized molecularly has 80.64 % pairwise similarity with Salmonella enterica strain Inspire 69 which has NCBI accession number JQ315905. The e value is 2.00 E-45. The PD/S10-6 has 99.64 % pairwise similarity with Escherichia coli strain EGE 4903307-101which has NCBI accession number KY655124 with the e value of 0. The 16SrRNA gene amplification of the bacteria isolates characterized molecularly at 1500bp. M is a 1Kbp DNA ladder. Lane B1 = P/D2 10-6, Lane B1 = P/S2 10-4.

The phylogenetic tree shown in figure four shows the Neighbor-joining phylogenetic dendrogram based on a comparison of the 16S rRNA gene sequences of the two Gram-negative representative isolates and some of their closest phylogenetic taxa. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Maximum Composite Likelihood method (Tamura et al., 2011) and are in the units of the number of base

substitutions per site. This analysis involved 7 nucleotide sequences. Codon positions included were 1st + 2nd + 3rd + Noncoding. All ambiguous positions were removed for each sequence pair (pairwise deletion option).

IV. CONCLUSION

The study above was based on isolation and molecular characterization of selected bacteria isolated from soil sample collected from pig house in Igboora. The study has been able to determines the population of microorganism (Total Coliform Count) of soil collected from pig house at the institution prevalence of some Staphylococcus aureus, Streptococcus spp, Neisseria spp, Enterococcus spp, Micrococcus spp, Escherichia coli, which occurred in higher frequencies. These organisms are opportunistic organisms that can be harmful to animal with unedifying health conditions and may also be pathogenic to human body. Based on the study, it could therefore be recommended that pig house should be fumigate once in a week to reduce the organism present in the soil and the pig's lives on top the soil should be given immunization against these microorganism present in the pig house.

V. REFERENCES

1. Hong Bai et al., "Spread of Airborne Antibiotic Resistance From Animal Farms to the Environment: Dispersal Pattern and Exposure Risk," *Environmental International*, vol. 158, pp. 1-1-12, 2022. Google Scholar | Publisher Link
2. Beata Trawińsk et al., "Effect of Pig Farm on Microbial Contamination of Soil," *Annals of Animal Science*, vol. 15, no. 1, pp. 165–175, 2015. Google Scholar | Publisher Link
3. J. Boes et al., "Survival of *Escherichia Coli* and *Salmonella Typhimurium* in Slurry Applied to Clay Soil on a Danish Swine Farm," *Preventive Veterinary Medicine*, vol. 69, no. 3-4, pp. 213–228, 2005. Google Scholar | Publisher Link
4. A. Buczyńska, and, Szadkowska-Stańczyk, "Occupational Hygiene and Health Hazards Related to Concentrated Animal Feeding Operations (in Polish)," *Medycyna Pracy*, vol. 61, pp. 323-331, 2010. Google Scholar | Publisher Link
5. D.G. Georgakopoulos et al., "Microbiology and Atmospheric Processes: Biological, Physical and Chemical Characterization of Aerosol Particles," *Biogeosciences*, vol. 6, no. 4, pp. 721–737, 2009. Google Scholar | Publisher Link
6. T.L. Gladding, "Concentration and Composition of Bioaerosol Emissions From Intensive Farms: Swine and Poultry Livestock," *Journal of Environmental Management*, vol. 272, 2020. Google Scholar | Publisher Link
7. M. Habteselassie et al., "Environmental Controls on the Fate of *Escherichia Coli* in Soil," *Water, Air and Soil Pollution*, vol. 190, pp. 143-155, 2008. Google Scholar | Publisher Link
8. Karin Hoelzer, Andrea Isabel Moreno Switt, and Martin Wiedmann, "Animal Contact as a Source of Human Nontyphoidal Salmonellosis," *Veterinary Research*, vol. 42, pp. 1-28, 2011. Google Scholar | Publisher Link
9. Jeroen L Kiers et al., "A High Molecular Weight Soluble Fraction of Tempeh Protects Against Fluid Losses in *Escherichia Coli*-Infected Piglet Small Intestine," *British Journal of Nutrition*, vol. 98, pp. 320-325, 2007. Google Scholar | Publisher Link
10. Julia G Kraemer et al., "Prevalence of Extended-Spectrum β-Lactamase Producing *Enterobacteriaceae* and Methicillin-Resistant *Staphylococcus Aureus* in Pig Farms in Switzerland," *Science of the Total Environment*, vol. 603, pp. 401-405, 2017. Google Scholar | Publisher Link
11. Bernhard Nowak et al., "Salmonella Contamination in Pigs at Slaughter and on the Farm: A Field Study Using an Antibody ELISA Test and a PCR Technique," *International Journal of Food Microbiology*, vol. 115, pp. 259-267, 2007. Google Scholar | Publisher Link
12. One Health Commission, "What Is One Health?" *One Health Commission*, 2018. [Online]. Available: https://www.onehealthcommission.org/en/why_one_health/what_is_one_health/
13. Susana Sanz et al., "Identification of *Enterococci*, *Staphylococci*, and *Enterobacteriaceae* from Slurries and Air in and Around Two Pork Farms," *Journal of Food Protection*, vol. 81, no. 11, pp. 1776-1782, 2018. Google Scholar | Publisher Link

14. Koichiro Tamura et al., "MEGA5: Molecular Evolutionary Genetics Analysis Using Maximum Likelihood, Evolutionary Distance, and Maximum Parsimony Methods," *Molecular Biology and Evolution*, vol. 28, no. 10, pp. 2731-2739, 2011. Google Scholar | Publisher Link
15. Qian Tang et al., "Distribution Characteristics of Bioaerosols Inside Pig Houses and the Respiratory Tract of Pigs," *Ecotoxicology and Environmental Safety*, vol. 212, pp. 1-9, 2021. Google Scholar | Publisher Link
16. Tongshuai Liu et al., "Characteristics of Aerosols from Swine Farms: A Review of the Past Two-Decade Progress," *Environment International*, vol. 178, pp. 1-22, 2023. Google Scholar | Publisher Link
17. Yingcai Wang, Yan Fu, Can Wang, and Nuanjia Wen, "Dissimilar Emission Characteristics between Bioaerosol and Suspended Particles From Gaseous Biofilters and Bioaerosol Health Risk Evaluation," *Aerosol and Air Quality Research*, vol. 18, pp. 1874-1885, 2018. Google Scholar | Publisher Link
18. Marcin Weiner, Jan Dacko, and Jacek Osek, "Molecular Analysis of Enterotoxigenic, Shigatoxigenic and Enteroaggregative *Escherichia Coli* Strains Isolated From Suckling Piglets With Diarrhoea by the Use of Pulsed-Field Electrophoresis," *Bulletin of the Veterinary Institute in Pulawy*, vol. 48, pp. 225–231, 2004. Google Scholar | Publisher Link