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Research Article

# Antimicrobial Spectrum, Growth/ killing kinetics, Conventional/Molecular assay and Ultraviolet Spectrophotometer Signatures of Characterizing Shigella Flexneri and Enterococcus Faecalis and Isolated from Swine House isolates

**Oludare Temitope Osuntokun**

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***Corresponding Author:** Department of Microbiology, Faculty of Science, Adekunle Ajasin University, Akungba Akoko. Ondo State Nigeria. E-mail: osuntokun4m@yahoo.com

## ABSTRACT

The objective of the study was to access microbial load and microorganism found in swine house depending on the sample site and to compare between the conventional and molecular methods (MEGA 6a rDNA sequencing) of characterization of swine house isolates. The antimicrobial spectrum, growth/ killing kinetics of the isolates using Ultraviolet spectrophotometer signatures were also evaluated. The sample were taken at the pig house from the wall [w] and at a distance of 2km and body[B] and floor[F] using sterile swap stick. The sample underwent serial dilution and a pure isolate was sub-cultured using nutrient agar and also biochemical test was conducted as a preliminary test. From the preliminary test, the following organism were identified, *Mycobacterium tuberculosis, Bacillus spp Bacillus anthracis, Bacillus cereus, Staphylococcus aureus, Clostridium sp.* In addition the samples were tested for antibiotics susceptibility test (Amtibiogram) using Kirby-bauer antibiotic susceptibility disc. All isolates were found to be susceptible to Ciprofloxacin, Levofloxacin, Gentamycin, Rifampicin, Streptomycin, Erythromycin and Amoxyl. Isolates were resistant to Norflaxacin, Chloramphenicol and Ampiclox. Molecular sequencing were performed on three isolates for a confirmatory test. It was observed that *Shigella flexneri and Enterococcus faecalis*. Growth rate and death rate / killing time of isolates using ultraviolet spectrophotometer from the swine house were measured. It was observed, At, wavelength 480λ. *Bacillus spp* has the highest growth rate of 0.525λ and *Bacillus subtilis* have the lowest growth rate of 0.001λ. At 84$^{th}$ hour, *bacillus spp* has the lowest death rate of 0.307 λ and *Bacillus cereus* have the highest death rate of 0.227λ, growth dynamic and killing time of bacteria isolates and addition of ciprofloxacin antibiotic at 24th hour using ultraviolet spectrophotometer. it was observed that at 0 hour, *Bacillus subtilis* has the highest growth rate of 0.251λ and *Bacillus cereus* have the lowest growth rate of 0.019λ. At the 84th hour, *Bacillus kaustophilus* has the lowest death rate of 0.152λ and *Bacillus subtilis* have the highest death rate of 0.097. Proper sanitation of pig house as well as the animals can help minimize the possible organisms found in the swine house which may serve as a major health hazards for people that consume pig and farmers in the pig house. It can also serve as food-borne pathogen posing potential health hazard when pork from infected animals are consumed.

**Keywords;** Conventional methods, Molecular Assay, Ultraviolet spectrophotometer, Swine House, Antimicrobial spectrum, Growth/ killing kinetics

## Introduction

Microbial load 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 (1). Studies determined the presence of numerous microorganisms in air of the swine facilities, the most frequently isolated bacteria included *Escherichia coli, Staphylococcus xylosus, Micrococcus lentus, Streptococcus uberis,* Leuconostoc lactis and *Shigella spp., Enterococcus faecium* and *Enterococcus faecalis* (2; 3). Besides, *Salmonella* rods were commonly identified among bacterial pathogens (4). Whereas (5) evaluating the samples taken from various sites in the swine unit (doors, floor, ventilation system, litter), recovered *Salmonella* rods in 70.7% of the samples. Bacterial pollution of air in different units of pig facility was studied by (6) who determined the highest total bacterial count (55600 per $m^3$ air) in the swine farrowing unit. 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 (7); (8). 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 (9).

Bacterial development and survival in soil is favoured by high temperature and moisture (10). (11) investigated seasonal changes in bacterial flora of manure from pigs before and after implementation of membrane filtration treatment. Microbes frequently isolated from animal feces, such as *Streptococcus spp* and *Lactobacillus* spp. dominated in July and October, *Clostridium* spp. in February and July, whereas *Corynebacterium* spp. in August and October. Substantial bacterial contamination also pertains to the area surrounding large scale livestock farms. (12) 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 (13). Microbial contamination of animal feces and natural environment, especially the presence of pathogenic bacteria, may pose human and animal health hazard. The above findings have prompted the evaluation of bacterial contamination of soil collected from a pig farm and manure from these animals depending on the season of the year and sampling sites. Microbiological homeostasis of the digestive tract ensures proper functioning of an animal's organism. Proper microbial activity adapted to the host ensures proper nutrient utilization and proper growth and development. Both enzymatic hydrolysis and microbiological decomposition of nutrients are important for the digestion of nutrients in pigs (14). Microorganisms inhabiting the gastrointestinal tract contribute to the production of vitamins and cofactors that decompose previously indigestible feed components, and bacteriostatic and antifungal substances, thus reducing populations of pathogenic microorganisms. It is important in animal production to maintain microbial homeostasis using various strategies, such as optimizing animal diets and ensuring appropriate zoo hygienic conditions. Suitable microbiota in the pig gastrointestinal tract can ensure increased health status and thus correct or increased production indices (15). Diet and its additives are the most controlled factors influencing the microbiological composition of the digestive system. Changes in the microbiota may by an increase in fiber content, as this is quite sensitive to its level in the feed ration of pigs (14). Then, xylanolytic and cellulolytic bacteria count is

subject to an increase. Changes also occur when zinc oxide (ZnO) is added to pig feed to prevent diarrhea. After analysis of the microbiome of pigs, changes at the genus level have been found. The number of bacteria of the genera Weissella, Leuconostoc, Streptococcus increase, while the number of Sarcina decrease significantly. As shown in these studies, component modifications in the diet cause changes in the microbiota of the gastrointestinal tract of pigs, which adapts to these changes in order to survive (16). A study carried out by (17) showed the influence of diatom the improvement of the health condition of pigs. A diet based on boiled white rice used in the study reduced the incidence of dysentery in pigs; however, increasing the amount of dietary fibre above 20% resulted in an increase in the count of *Brachyspirahyo dysenteriae* and an increase in the risk of dysentery. Positive effects were also observed when the diet was fermented and supplemented with an organic acid, i.e., lactic acid (18). In the case of pigs, stress is another factor that significantly affects the microbiological composition of the digestive system. This may be caused by many factors, which include weaning, transport and feed reduction (19). In the case of weaning, the number of *Lactobacillus* increases and *Bifidobacterium* and *E. coli* decrease. The dominant bacteria are *L. sobrius* and *L. amylovorus*. The relative stability of microbiota starts around 11 days after weaning (15). In the case of transport stress, however, the population of *Salmonella typhimurium* in fecal samples of pigs hosting these bacteria is subject to an increase. A significant level of these bacteria was found in pigs fed before transport to the slaughterhouse, but it was not demonstrated when feed was withdrawn 24h before slaughter (10). The development and subsequent stability of gastrointestinal microorganisms is essential for the normal dietary, physiological and immunological functions of pigs. Disturbances in the intestinal microbiome create an opportunity for pathogenic microorganism development which may result in increased disease occurrence. Common management practices in intensive pig production, such as early and sudden weaning, poor hygiene, and prophylactic use of antibiotics, can caused is turbances in the intestinal microbial ecosystem, exposing animal sto the development of pathogens and consequently diseases. The microbiota of the digestive system not only play a protective role, but can also influence the production parameters of pigs. Changes in the microbiota of the digestive system affect assimilation of nutrients, and in the case of fattener pigs, also the quality and quantity of meat. The study by (9) showed changes in the composition of microorganisms at the genus level between individuals characterized by high meat quality and those with low meat quality.

# MATERIALS AND METHODS

## COLLECTION OF SAMPLES

The samples were collected from Animal farm from Adekunle Ajasin University, Akungba-Akoko, Ondo State, Nigeria with a swap stick and sterile water. All samples were transported to the Department of` Microbiology Laboratory at Adekunle Ajasin University Akungba-Akoko, Ondo state for microbial analysis.

## SERIAL DILUTION OF ISOLATES FROM SWINE HOUSE

Serial dilution was carried out on the swine house samples collected. 9mls of distilled water was measured using sterile syringe and needle into ten(6) test tubes and corked with cotton wool and covered with aluminum foil and sterilized in an autoclave at $121^0$C for 15 minutes in order to prevent contamination. After sterilization the water was allowed to cool to about $35^0$C - $40^0$C. 1g of the macerated root sample was measured and dispersed into the 9mL of water contained in the first test tube making $10^{-1}$ dilution using a sterile syringe and needle, from $10^{-1}$ dilution, aliquots of 1mL into the second test tube containing 9mL of sterile water making $10^{-2,}$ this process was repeated for other test tubes until the $10^{-6}$test tube was prepared(20).

## ISOLATION OF ISOLATES FROM SWINE HOUSE

The samples collected were separately washed with tap water, followed by surface sterilization using 70% ethanol for 30 seconds, 2% Sodium hypochlorite (NaOCl) for 5 minutes, 3% Hydrogen peroxide for 30 seconds and then rinsed five times with distilled water, to remove epiphytic microbes. Ten grams of these samples were cut to 2-3 cm pieces and macerated using sterilized mortar and pestle with 12.5 mm potassium phosphate buffer (pH 7.1), followed by a 10-fold serial dilution where 0.5ml of the $10^{-3}$ dilution was plated using the pour plate method on Nutrient Agar supplemented with cycloheximide (100 µg/mL) to inhibit fungal growth. Inoculated Petri plates were incubated at 37°C for 24 hours. After the incubation time, the colony forming units (CFU) for each plate was estimated (20). Isolates differing in morphological appearance were selected and were streaked onto new plates until pure cultures were obtained. Pure cultures of bacterial isolates were maintained on NA slants and were stored at 4°C(21).

## POUR PLATE TECHNIQUE FOR ENUMERATION OF BACTERIA COUNT

From the serial dilution above, 0.5mL of the $10^{-3}$and $10^{-5}$from each samples was spread evenly into labelled Petri-dishes respectively using Nutrient Agar. The prepared agar (NA) was allowed to cool to $45^0$C and aseptically poured into the Petri-dishes (about 20mL each) to make a plate. The plates was rocked to ensure even distribution of the sample within the medium and also to allow the agar form a uniform layer(21).

## INCUBATION OF ISOLATES FROM SWINE HOUSE

After the agar has been cooled and gelled, the Petri-dishes was inverted to prevent condensation dropping from the lid into the agar and then incubated in the incubator at $37^0$C for 24-78 hours.

## TOTAL BACTERIA COUNT OF ISOLATES FROM SWINE HOUSE

After 24 to72 hours of incubation, the plates were examined thoroughly and the plates containing 10-7 was discarded due to lack of distinct colony, other plate used in microbiological culture of 10-10 was properly observed. The total colonies was counted using a colony counter. The count can also be done easily by dividing the plate into four quarters, the first quarter counted first and recorded then the second quarter till the fourth quarter which was noted and recorded(21).

## IDENTIFICATION OF SWINE HOUSE ISOLATES

Preliminary identification of the bacterial isolate where based on their morphological characteristics and result from various biochemical tests carried out on them. Each of the isolates were cultured on media and observed after 24h of incubation for their morphological characteristics. Biochemical characteristics were determined according to the methods of Olutiola (22) and examined according to the Bergey's Manual of Determinative Bacteriology (22). This was done to confirm the identity of the swine house bacteria isolates.

## ISOLATION OF ISOLATES FROM SWINE HOUSE SAMPLES ISOLATION METHOD (Sub-Culturing)

Nutrient agar was prepared according to manufacturer instruction. The homogenized agar was poured into sterilized McCartney bottles and autoclaved at $121^0$C for 15 minutes. The bottles containing media was placed in slanting position and allowed to cool. After cooling, distinct colonies was sub-cultured by streaking on the slants. This was done by using sterile inoculating loop to pick part of the colony and inoculated on the solidified media in the bottle (slant). After the streaking process, the slants was incubated at $37^0$C for 24 hours. The slants will further be kept in the refrigerator to serve as stock culture for subsequent test during identification. This process was carried out aseptically to avoid contamination(21).

## MORPHOLOGICAL CHARACTERIZATION OF ISOLATES FROM SWINE HOUSE

The appearance of the colony of each isolate on the agar media was studied and characteristics such as shape, edge, pigment, opacity, elevation and surface were observed as described by Olutiola (23).

## GRAM STAINING OF ISOLATES FROM SWINE HOUSE

This test was carried out on 24 h old cultures of the isolate in order to determine their gram reaction and cellular morphology. The gram reaction differentiates bacteria into gram positive and gram-negative bacteria.

A smear of the culture between 18 to 24 hours was prepared on a clean grease free microscope slide with a drop of sterile distilled water and mixed. The smear was allowed to dry and then heat-fixed by passing the slide over a spirit flame once or twice. The heat-fixed smear was flooded with crystal violet and allowed to stain for 60s after which the stain was poured off and rinsed with water, the slide was flooded with iodine solution and will be allowed to stand for another 60s and then poured off and rinsed with water, the smear was decolorized with 95% ethanol and rinsed immediately after 10s with water. The smear was further counter stained with safranin for another 60s after which was gently rinsed off with water. It was air-dried and examined under the oil immersion objectives of 100x light microscope. The gram reaction, shape and arrangement of cell were then recorded (24).

## BIOCHEMICAL TEST FOR IDENTIFICATION OF THE SWINE HOUSE ISOLATES.

## CATALASE TEST

The inability of strict anaerobes to synthesize catalase, peroxidase, or superoxide dismutase may explain why oxygen is poisonous to these microorganisms. In the absence of these enzymes, the toxic concentration of $H_2O$ cannot be degraded when these organisms are cultivated in the presence of oxygen. Organism capable of producing catalase rapidly degrade hydrogen peroxide which is a tetramer containing four porphyrin heme groups (i.e. iron groups) that will allow the enzyme to react with hydrogen peroxide. The enzyme catalase is present in most cytochrome containing aerobic and facultative anaerobic bacteria. Catalase has one of the highest turnover numbers of all enzyme such that one molecules of catalase can convert millions of molecules of hydrogen peroxide to water and oxygen in a second. Catalase production and activity can be detected by adding the substrate $H_2O_2$ to an approximately incubated (18-to 24-h) slant culture. Organisms which produces the enzyme break down the hydrogen peroxide, and the resulting oxygen production produces bubbles in the reagent drop, indicating a positive test. Organisms lacking the cytochrome system also lack the catalase enzyme and are unable to break down hydrogen peroxide, into $O_2$ and water and thus are catalase negative(24).

## OXIDASE TEST

This was carried out using oxidase reagent as described by Baker *et al.* (25). Two drops of freshly-prepared oxidase reagent (1% aqueous tetramethyl-p-phenylenediamine hydrochloride solution) were placed on a piece of filter paper. A part of the colony of the bacterial isolate was collected using one end of sterile grease free glass slide and smeared across the filter paper impregnated with the oxidase reagent and observed for deep purple colour within 10s.

## CITRATE TEST

The agar used in citrate test is Simmon's Citrate Agar, it is used to test an organism ability to utilize citrate as a source of energy. The medium contain citrate as the sole carbon source and inorganic ammonium salts ($NH_4HPO_4$) as the source of nitrogen. It was done by dissolving the agar and gently heat with mixing and boiling until it dissolved. A 5 ml was dispensed into each tube and autoclaved at 121°C for 15 min. It was

cooled and slanted before the test organism was streaked with a light inoculum. It was then be incubated aerobically at 37$^{o}$C for 5 days and examined. Those that changed from original colour (green) to blue or yellow were considered positive. While those that retained the green colour were considered negative. Uninoculated control tubes were included then the results were then recorded (24).

## INDOLE TEST

Some microbes are capable of hydrolyzing the amino acid tryptophan and one of the end products is indole. The latter reacts with 4-dimethyl amino benzy-aldehyde to form a dark red dye. Each of the bacterial isolates were cultured in sterile nutrient broth for 48h at 37$^{0}$C. 2mls of chloroform were added to the broth culture and mixed gently. About 2mls of Kovac's reagent were later added, shaken gently and allowed to stand for 20min. a red colour at the reagent layer indicates indole production (26). The results were then recorded.

## SUGAR FERMENTATION TEST

A situation whereby carbohydrates are utilized in the partial or total absence of oxygen is referred to as fermentation. But sugar is utilized in the presence of oxygen such reactions are known as oxidation. Sterilization of the basal medium was done using an autoclave at 121$^{0}$C for 15min. Ten percent (10%) sterile solution of the test sugar (glucose, fructose, mannitol, dextrose and galactose) was added, inverted Durham tubes was put into each tube. Different isolates were inoculated into each test tube according to the labeling using a sterile inoculating loop. Un-inoculated tubes serve as controls. The results were examined daily for up to 7days in which methyl red indicator changed to yellow. A yellow coloration indicated growth and acid production. Also, the upper part of the Durham tubes was examined to detect any accumulated gas which indicated gas production. (24) results were then recorded.

## HEMOLYSIS TEST

Nutrient agar was prepared and autoclaved at 121$^{0}$C for 15 minutes, it was then allowed to cool. 5ml of blood was added to the sterile nutrient agar to prepare blood agar. The prepared blood agar was poured into sterile petri dishes and allow to set. Colony was picked from the stocked culture and streaked on the blood agar, it was then incubated at 37$^{0}$C for 24 hours. After incubation, the results indicates an alpha-hemolysis, beta-hemolysis and gamma-hemolysis. Alpha hemolysis is indicated by a greenish-grey or brownish discoloration around the colony as a result of the partial lysis of the red blood cells. Beta hemolysis is indicated by a clear zone of hemolysis under and around the colonies when grown on blood agar and this clear zone appears as a result of the complete lysis of the red blood cells present in the medium, causing denaturation of hemoglobin to form colorless products. Gamma hemolysis which is also refers to as non-hemolysis as there is no lysis of red blood cells occurs as a result, no change of coloration or no zone of hemolysis. (27).

## SUCROSE FERMENTATION TEST

3g of peptone powder was dissolved in 180ml of distilled water in appropriately labelled conical flask and 0.5g of phenol red was added. 1g of Sucrose sugar was added into the conical flask and shaken thoroughly. The solution was dispensed in 5ml amounts into test tubes with inverted Durham's tubes and autoclaved for 15minutes. The test tubes were then inoculated with loop full of test organisms and incubated at 37 for maximum of 48 hours. The test was observed for acid production leading to colour change (red to yellow) as well as gas production that causes the displacement of the liquid in the inverted Durham's tubes which indicates a positive test. (27).

## LACTOSE: FERMENTATION TEST

3g of peptone powder was dissolved in 180ml of distilled water in appropriately labelled conical flask and 0.5g of phenol red was added. 1g of lactose sugar

was added into the conical flask and shaken thoroughly. The solution was dispensed in 5ml amounts into test tubes with inverted Durham's tubes and autoclaved for 15minutes. The test tubes were then inoculated with loop full of test organisms and incubated at 37 for maximum of 48 hours. The test was observed for acid production leading to colour change (red to yellow) as well as gas production that causes the displacement of the liquid in the inverted Durham's tubes which indicates a positive test. (27).

## MOTILITY TEST

A little immersion oil was placed round the edge of a cavity slide and then a loopful of the bacterial colony was transferred to the centre of a clean dry cover slip with a sterile inoculating lop. The cavity slide was inverted over the cover slip such that the culture drop was in the centre of the slide depression. The culture drop appeared hanging. This was examined immediately for motility under the oil immersion microscope(28).

## METHYL RED TEST

Using a Pasteur's pipette, 10 drops of methyl red pH indicator was added to each tube and the tube was swirled gently to mix the drops into the broth. Each tube was examined for color change. Bacteria that produce many acids from the breakdown of dextrose(glucose) in the MR-VP medium cause the pH to drop to 4.2. At this pH, methyl red becomes red, a red color represents a positive test. Bacteria that produce fewer acids from the breakdown of glucose drop at pH to 6.0. At this pH, methyl red is yellow and this represents a negative test(28).

## VOGES-PROKAUER TEST

This test was carried out by inoculating MR-VP medium and incubating at 37°c for 2 days. It was then tested with methyl red and then 0.6mL of alpha naphthol sodium(about 15 drops) and 0.2mL of 40% KOH (about 10 drops) was added. It was shakes and examined for the red colour of a positive reaction after 15 minutes to 1hour. A positive reaction developed a red color after 15-60min. Under alkaline conditions and in the presence of oxygen, acetyl-methyl-ethanol will be oxidized to diacetyl which reacts with creatine to give a red colour, creatine is present in peptone(28).

## ANTIBIOTIC SUSCEPTIBILITY TEST FOR THE SWINE HOUSE ISOLATES

The test was performed to determine the phenotypic resistant of the bacterial isolates to commonly used antibiotics. These tests were carried out following the Kirby-Bauer disc diffusion method of (CLSI, 2009). Inoculum from culture of bacteria isolates on nutrient agar slants were inoculated into test tubes containing sterilized nutrient broth and incubated at $37^0$C for 18h which serve as the stock for the test. Mueller-Hinton agar was prepared and sterilized, then dispensed into sterilized Petri dishes. The plates were allowed to cool for about 15min so as to allow it to gel and excess surface moisture to be absorbed. The inoculum was introduced into plates by streaking before applying the antibiotics impregnated discs. Multi-test Predetermined commercial Gram negative and Gram positive discs were applied to the surface of the well labeled inoculated agar plated aseptically using sterile forceps. The discs were then placed firmly by slightly pressing on the inoculated plates with the sterilized forceps to ensure complete contact with the agar. After 24h of incubation, each plates was examined, susceptibility to each antibiotics were indicated by a clear zone. The zone of inhibition were measured using a calibrated ruler was held on the back of the inverted petri plate and was recorded (29).

# CONFIRMATORY MOLECULAR CHARACTEIZATION OF SWINE HOUSE ISOLATES

## BACTERIA DNA EXTRACTION OF ISOLATES FROM SWINE HOUSE

DNA was extracted using the protocol stated by (1). Briefly, Single colonies grown on medium were transferred to 1.5 ml of liquid medium and cultures were grown on a shaker for 48 h at 28ºC. After this period, cultures were

centrifuged at 4600g for 5 min. The resulting pellets were resuspended in 520 µl of TE buffer (10 mM Tris-HCl, 1mM EDTA, pH 8.0). Fifteen microliters of 20% SDS and 3 µl of Proteinase K (20 mg/ml) were then added. The mixture was incubated for 1 hour at 37ºC, then 100 µl of 5 M NaCl and 80 µL of a 10% CTAB solution in 0.7 M NaCl were added and votexed. The suspension was incubated for 10 min at 65 ºC and kept on ice for 15 min. An equal volume of chloroform: isoamyl alcohol (24:1) was added, followed by incubation on ice for 5 min and centrifugation at 7200g for 20 min. The aqueous phase was then transferred to a new tube and isopropanol (1: 0.6) was added and DNA precipitated at –20ºC for 16 h. DNA was collected by centrifugation at 13000g for 10 min, washed with 500 µl of 70% ethanol, air-dried at room temperature for approximately three hours and finally dissolved in 50 µl of TE buffer(20).

## BACTERIA POLYMERASE CHAIN (PCR) REACTION OF ISOLATES FROM SWINE HOUSE

PCR sequencing preparation cocktail consisted of 10 µl of 5x Go Taq colourless reaction, 3 µl of 25mM MgCl2, 1 µl of 10 mM of dNTPs mix, 1 µl of 10 pmol each 27F 5'- AGA GTT TGA TCM TGG CTC AG-3' and - 1525R, 5′-AAGGAGGTGATCCAGCC-3′ primers and 0.3units of Taq DNA polymerase (Promega, USA) made up to 42 µl with sterile distilled water 8µl DNA template. PCR was carried out in a GeneAmp 9700 PCR System Thermalcycler (Applied Biosystem Inc., USA) with a Pcr profile consisting of an initial denaturation at 94°C for 5 min; followed by a 30 cycles consisting of 94°C for 30 s, 50°C for 60s and 72°C for 1 minute 30 seconds ; and a final termination at 72°C for 10 mins. And chill at 4oC.GEL(20).

## MOLECULAR INTEGRITY OF ISOLATES FROM SWINE HOUSE

The integrity of the amplified gene fragments was checked on a 1.5% Agarose gel ran to confirm amplification. The buffer (1XTAE buffer) was prepared and subsequently used to prepare 1.5% agarose gel. The suspension was boiled in a microwave for 5 minutes. The molten agarose was allowed to cool to 60°C and stained with 3µl of 0.5 g/ml ethidium bromide (which absorbs invisible UV light and transmits the energy as visible orange light). A comb was inserted into the slots of the casting tray and the molten agarose was poured into the tray. The gel was allowed to solidify for 20 minutes to form the wells. The 1XTAE buffer was poured into the gel tank to barely submerge the gel. Two microliter (2 l) of 10X blue gel loading dye (which gives colour and density to the samples to make it easy to load into the wells and monitor the progress of the gel) was added to 4µl of each PCR product and loaded into the wells after the 100bp DNA ladder was loaded into well 1. The gel was electrophoresed at 120V for 45 minutes visualized by ultraviolet trans-illumination and photographed. The sizes of the PCR products were estimated by comparison with the mobility of a 100bp molecular weight ladder that was ran alongside experimental samples in the gel(20).

## PURIFICATION OF AMPLIFIED PRODUCT

After gel integrity, the amplified fragments were ethanol purified in order to remove the PCR reagents. Briefly, 7.6 µl of Na acetate 3M and 240 µl of 95% ethanol were added to each about 40µl PCR amplified product in a new sterile 1.5 µl tube eppendorf, mix thoroughly by vortexing and keep at -20°C for at least 30 min. Centrifugation for 10 min at 13000 g and 4°C followed by removal of supernatant (invert tube on trash once) after which the pellet were washed by adding 150 µl of 70% ethanol and mix then centrifuge for 15 min at 7500 g and 4°C. Again remove all supernatant (invert tube on trash) and invert tube on paper tissue and let it dry in the fume hood at room temperature for 10-15 min. then resuspend with 20 µl of sterile

distilled water and kept in -20oC prior to sequencing. The purified fragment was checked on a 1.5% Agarose gel ran on a voltage of 110V for about 1hr as previous, to confirm the presence of the purified product and quantified using a nanodrop of model 2000 from thermo scientific(20).

## MOLECULAR MEGA6A rDNA SEQUENCING

The amplified fragments were sequenced using a Genetic Analyzer 3130xl sequencer from Applied Biosystems using manufacturers' manual while the sequencing kit used was that of BigDye terminator v3.1 cycle sequencing kit. Bio-Edit software and MEGA 6 were used for all genetic analysis(20).

## ULTRAVIOLET SPECTROPHOTOMETER SIGNATURES (DETERMINATION OF Growth Dynamic and Death Rate (KILLING KINECTICS)OF ISOLATES

Growth dynamic refers to the rate at which cells of microorganism grow at a given time. This test was done to determine the rate of growth of the isolates as well as their killing time in due time. Colony was picked from the stocked culture slant and inoculated into nutrient broth which was incubated for 24hours at $37^0$C. A loopful of organism was picked from the broth culture into nutrient broth in three sets which are set A, B, and C respectively. Ultraviolet spectrophotometer was set at 480λ wavelength, warmed up for 15 minutes and then the control was first read, the first was reading was taken at zero hour and it continues after every 12 hours for 6 times. At the 3rd reading, which is the 24th hour of set B, ciprofloxacin was added to determine up the rate of kill. (30).

## RESULTS

The organisms were isolated using both microscopic and macroscopic examination and preliminary isolation methods were used which was further subjected to preliminary biochemical test and the sugar fermentation of some simple sugars were carried out to identify the isolated organisms ,the growth and death rate were assayed using ultra violet spectrophotometer. Confirmatory identification of the isolated organisms were further carried out using Molecular sequencing which gives a distinct family and sub-species of the isolated organisms.

**Table 1:** Shows the type of swine house bacterial isolates collected, the numbers of plant sample collected, place of collection and time of collection. In this table, the type of sample collected were from the Animal house floor, Animal body and Animal house wall, the number of sample collected was 9 and the samples were collected at the Aaua farm spot 1, 2 and 3 at Adekunle Ajasin University, Akungba Akoko, at 8:00A.M in the Morning.

**Table 2:** this table shows the dilution factor and the number of colony found on each plate after incubation at 37% for 24hours, on the wall, body and floor. It was observed that the plates containing the lowest dilution factor($10^{-3}$) has the highest number of colonies while the plates with the highest dilution factor($10^{-5}$) has the lowest number of colonies.

**Table 3**: this table shows the samples and their cultural characteristics and also the date of collection. After incubation, it was observed that S1F$10^{-3}$, SIB$10^{-5}$ and S3W $10^{-3}$ shows a cultural characteristics as rise, light, creamy and smooth edges. S1W $10^{-3}$, SIB $10^{3}$, and S1F $10^{-5}$ shows raise, cream and rough edges. S3B $10^{-5}$, S3B $10^{-3}$, S2B $10^{-3}$, S1W $10^{-5}$, S3W $10^{-5}$, S2F $10^{-3}$, S2F $10^{-5}$ and S3F $10^{-5}$ shows raise, cream and smooth edges. S2W $10^{-3}$, S2B $10^{-5}$ and S3F $10^{-3}$ shows raise, light, cream and rough edges. Lastly S2W $10^{-5}$ shows raise, cream and smooth edges.

**Table 1**: Sample collected, number of sample collected, place and time of collection

| Sample collected | Number of samples collected | Place of collection | Collection time |
|---|---|---|---|
| ANIMAL HOUSE FLOOR | 3 | AAUA FARM SPOT 1 | 8:00 A.M |
| ANIMAL BODY | 3 | AAUA FARM SPOT 2 | 8:00 A.M |
| ANIMAL HOUSE WALL | 3 | AAUA FARM SPOT 3 | 8:00 A.M |

**Fig 1;Colony Count and Dilution Factors of Bacterial Isolates From Floor, Body and Wall of Swine House On Nutrient Agar**

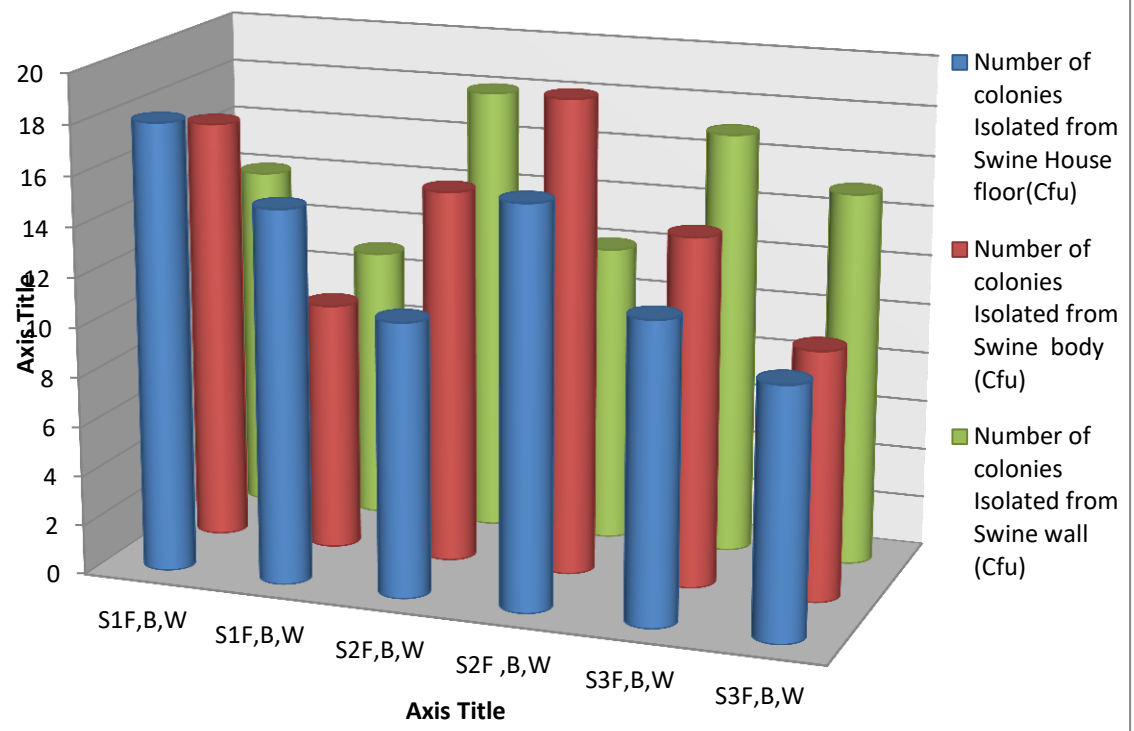


**Table 2**: Colony Count and Dilution Factors of Bacterial Isolates From Floor, Body and Wall of Swine House On Nutrient Agar

| Sample code (Swine House floor) | Dilution factor | Number of colonies Isolated from Swine House floor(Cfu) | Sample code (Swine Body Isolates) | Dilution factor | Number of colonies Isolated from Swine body (Cfu) | Sample code (Swine House Wall) | Dilution factor | Number of colonies Isolated from Swine wall (Cfu) |
|---|---|---|---|---|---|---|---|---|
| S1F | $10^{-3}$ | 18 | S1B | $10^{-3}$ | 17 | S1W | $10^{-3}$ | 14 |
| S1F | $10^{-5}$ | 15 | S1B | $10^{-5}$ | 10 | S1W | $10^{-5}$ | 11 |
| S2F | $10^{-3}$ | 11 | S2B | $10^{-3}$ | 15 | S2W | $10^{-3}$ | 18 |
| S2F | $10^{-5}$ | 16 | S2B | $10^{-5}$ | 19 | S2W | $10^{-5}$ | 12 |
| S3F | $10^{-3}$ | 12 | S3B | $10^{-3}$ | 14 | S3W | $10^{-3}$ | 17 |
| S3F | $10^{-5}$ | 10 | S3B | $10^{-5}$ | 10 | S3W | $10^{-5}$ | 15 |

KEY: S1F: Section 1 Floor, S2F: Section 2 Floor, S3F: Section 3 Floor,
KEY: S1B: Section 1 Body, S2B: Section 3 Body, S3B: Section 3 Body
KEY: S1W: Section 1 Wall, S2W: Section 2 Wall, S3W: Section 3 Wall

**Table 3**: This table shows the samples and their cultural characteristics and also the date of collection. After incubation, it was observed that S1F$10^{-3}$, SIB$10^{-5}$ and S3W $10^{-3}$ shows a cultural characteristics as raise, light, creamy and smooth edges. S1W $10^{-3}$, SIB $10^{-3}$, and S1F $10^{-5}$ shows raise, cream and rough edges. S3B $10^{-5}$, S3B $10^{-3}$, S2B $10^{-3}$, S1W $10^{-5}$, S3W $10^{-5}$, S2F $10^{-3}$, S2F $10^{-5}$ and S3F $10^{-5}$ shows raise, cream and smooth edges. S2W 10-3, S2B 10-5 and S3F 10-3 shows raise, light, cream and rough edges. Lastly S2W $10^{-5}$ shows raise, cream and smooth edges.

**Table 4**: This table shows the morphological characteristics of the isolates and the date of observation. The physical appearance was observed after 24hours of incubation at 37%. It was noticed that S1B $10^{-3}$, S1B $10^{-5}$, S2B $10^{-5}$, S3B $10^{-3}$, S3B $10^{-5}$, S1W $10^{-3}$, S1W $10^{-5}$, S2W $10^{-3}$, S1F $10^{-3}$, S1F $10^{-5}$, S2F $10^{-3}$, S2F $10^{-5}$, S3F $10^{-3}$, and S3F $10^{-5}$ appears milk, circular edges, opaque and not clear. and S2B $10^{-3}$ and S2W $10^{-5}$ appears yellow, circular edges, opaque and not clear while S3W $10^{-3}$ and S3W $10^{-5}$ appears milk, irregular edges, opaque and not clear. This shows the variation in the physical appearance of the pure isolates.

**Table 5**: This shows the gram staining results of all isolates after observation under the electron microscope. After viewing under the microscope, it was observed that all the isolate was gram positive. The floor isolates from section two dilution factor $10^{-5}$, section three dilution factor $10^{-3}$ and $10^{-5}$ are short rod while the body isolates from section two dilution factor $10^{-5,}$ section 3 dilution factor $10^{-3}$ and $10^{-5}$ are long rod, body isolates from section two dilution factor $10^{-3}$ is short rod. And also wall isolates from section two dilution factor $10^{-3}$ and $10^{-5}$ are short rod while section three dilution factor $10^{-3}$ and$10^{-3}$ are long rod.

**Table 6**: Shows the biochemical tests carried on the recovered bacterial isolates, the tests include; motility, urease, indole, It was observed in this table that , S2F$^{-3}$, S3F$^{-5}$, S3B$^{-3}$, S3B$^{-5}$ was negative to motility test while other isolates were positive to motility test. It was also observed that , S2F$^{-3}$ , S3F$^{-3}$, S3F$^{-5}$, S3B$^{-5}$, S2B$^{-5}$, S3W$^{-3}$ was negative to indole test, while other isolates were positive to indole test. It was observed that S3F$^{-3}$, S2W$^{-5}$ were negative to urease test while other isolates were positive to urease test. oxidase, catalase, methyl- red, voges prokaeur test, hemolysis, citrate and

identification of the isolated organisms. In this table, it was observed that $S2B^{-5}$, $S3B^{-3}$, $S2W^{-5}$ were negative to oxidase test, while other isolates were positive to oxidase test. It was also observed that $S3F^{-5}$, $S2B^{-5}$, $S2B^{-3}$ were positive to catalase test while other isolates were negative to catalase test. It was further observed that all bacterial isolates were positive to citrate test except $S2F^{-3}$, $S2B^{-3}$. It was also observed that all bacterial isolates were all negative to methyl-red test. It was also observed that $S2F^{-3}$, $S3F^{-3}$, $S3B^{-5}$, $S3W^{-3}$, $S2W^{-5}$ were negative to voges-prokaeurs test while other isolates were positive to voges-prokaeur test. It was also observed that all bacterial isolates were positive to citrate test.

**Table 7**: Shows the fermentation tests performed on the bacterial isolates, the tests include; Lactose, Sucrose, Dextrose, sugar fermentation, gas production and hydrogen sulphide tests. It was observed that $S2B^{-5}$, $S2W^{-3}$ isolates were positive to hydrogen sulphide test while other isolates were negative to hydrogen sulphide test. It was observed that all bacterial isolates were negative to gas production test, it was observed that , $S2F^{-3}$, $S3W^{-3}$ and $S3W^{-3}$ are negative to Lactose, Sucrose and Dextrose sugar respectively while other bacterial isolates were positive to Lactose, Sucrose and Dextrose sugar respectively.

**Table 8**: The isolated organisms include *Mycobacterium tuberculosis, Bacillus spp(3), Bacillus anthracis(2), Bacillus cereus, Bacillus subtilis, Staphylococcus aureus(2), Clostridium spp.*

**Table3**: cultural Characteristic (Macroscopic examination)of Bacterial Isolates From Floor, Body and wall of Swine House On Nutrient Agar

| Sample code (Swine House floor) | Cultural characteristics | Sample code | Cultural characteristics | Sample code | Cultural characteristics |
|---|---|---|---|---|---|
| $S1F\ 10^{-3}$ | Raise, light, creamy, smooth edges | $S1B\ 10^{-3}$ | Raise creamy, rough edges | $S1W10^{-3}$ | Raise creamy, rough edges |
| $S1F\ 10^{-5}$ | Raise ,creamy, rough edges | $S1B\ 10^{-5}$ | Raise light, creamy, smooth edges | $S1W\ 10^{-5}$ | Raise creamy, smooth edges |
| $S2F\ 10^{-3}$ | Raise, creamy, smooth edges | $S2B\ 10^{-3}$ | Raise creamy, smooth edges | $S2W\ 10^{-3}$ | Raise light, creamy, rough edges |
| $S2F\ 10^{-5}$ | Raise, creamy, smooth edges | $S2B\ 10^{-5}$ | Raise light, creamy, rough edges | $S2W\ 10^{-5}$ | Raise creamy, smooth edges |
| $S3F\ 10^{-3}$ | Raise, light, creamy, rough edges | $S3B\ 10^{-3}$ | Raise creamy, smooth edges | $S3W\ 10^{-3}$ | Raise light, creamy, smooth edges |
| $S3F\ 10^{-5}$ | Raise, creamy, smooth edges | $S3B\ 10^{-5}$ | Raise creamy, smooth edges | $S3W\ 10^{-5}$ | Raise creamy, smooth edges |

KEY: S1F: Section 1 Floor, S2F: Section 2 Floor, S3F: Section 3 Floor,
KEY: S1B: Section 1 Body, S2B: Section 3 Body, S3B: Section 3 Body
KEY: S1W: Section 1 Wall, S2W: Section 2 Wall, S3W: Section 3 Wall

**Table 4**; Morphological Characteristics of Sub-Cultures Bacterial Isolates From Floor, Body and Wall of Swine House On Nutrient Agar

| Sample code (Swine House floor) | Morphological characteristics | Sample Code (Body) | Morphological characteristics | Sample code (Wall) | Morphological characteristics |
|---|---|---|---|---|---|
| S1F x $10^{-3}$ | Milk, circular, edges, opaque, not clear | S1B x $10^{-3}$ | Milk, circular edges, opaque, not clear | S1W x $10^{-3}$ | Milk, circular edges, opaque, not clear |
| S1F x $10^{-5}$ | Milk, circular edges, opaque, not clear | S1B x $10^{-5}$ | Milk, circular edges, opaque, not clear | S1W x $10^{-5}$ | Milk, circular edges, opaque, not clear |
| S2F x $10^{-3}$ | Milk, irregular, edges, opaque, translucent | S2B x $10^{-3}$ | Yellow, circular edges, opaque, not clear | S2W x $10^{-3}$ | Milk, circular, edges, opaque, Translucent |
| S2F x $10^{-5}$ | Milk, circular edges, opaque, translucent | S2B x $10^{-5}$ | Milk, circular edges, opaque, not clear | S2W x $10^{-5}$ | Yellow, circular edges, opaque, not clear |
| S3F x $10^{-3}$ | Milk, irregular edges, opaque, not clear | S3B x $10^{-3}$ | Milk, circular edges, opaque, translucent | S3W x $10^{-3}$ | Milk, irregular edges, opaque, not clear |
| S3F x $10^{-5}$ | Milk, circular edges, opaque, not clear | S3B x $10^{-5}$ | Milk, circular edges, opaque, not clear | S3W x $10^{-5}$ | Milk, irregular edges, opaque, not clear |

KEY: S1F: Section 1 Floor, S2F: Section 2 Floor, S3F: Section 3 Floor,
KEY: S1B: Section 1 Body, S2B: Section 3 Body, S3B: Section 3 Body
KEY: S1W: Section 1 Wall, S2W: Section 2 Wall, S3W: Section 3 Wall

**Table 5** Gram staining (Microscopic examination) of isolates From Floor, Body and Wall of Swine House

| Sample code(Swine House floor) | Grams stain | Shape |
|---|---|---|
| S2F x $10^{-3}$ | + | Short rod |
| S3F x$10^{-3}$ | + | Short rod |
| S3F x $10^{-5}$ | + | Short rod |
| S2B x $10^{-3}$ | + | Short rod |
| S2B x $10^{-5}$ | + | Long rod |
| S3B x $10^{-5}$ | + | Long rod |
| S3B x $10^{-3}$ | + | Long rod |
| S3W x $10^{-5}$ | + | Long rod |
| S3W x $10^{-3}$ | + | Long rod |
| S2W x $10^{-3}$ | + | Short rod |
| S2W x $10^{-5}$ | + | Long rod |

**Table 6**; Biochemical Tests for bacteria identification of isolates From Floor, Body and Wall of Swine House

| Sample code (Swine House floor) | Motility | Indole | Urease | Oxidase | Catalase | Methyl Red | Voges-prokaeur | Hemolysis | Citrate |
|---|---|---|---|---|---|---|---|---|---|
| S2F x $10^{-3}$ | -ve | -ve | +ve | +ve | -ve | -ve | -ve | Gamma | +ve |
| S3F x $10^{-3}$ | +ve | -ve | -ve | +ve | -ve | -ve | -ve | Beta | -ve |
| S3F x $10^{-5}$ | -ve | -ve | +ve | +ve | +ve | -ve | +ve | Beta | +ve |
| S2B x $10^{-3}$ | +ve | -ve | +ve | -ve | +ve | -ve | +ve | Beta | -ve |
| S3B x $10^{-3}$ | +ve | +ve | +ve | +ve | +ve | -ve | +ve | Beta | +ve |
| S3B x $10^{-5}$ | -ve | -ve | +ve | -ve | -ve | -ve | +ve | Beta | +ve |
| S3B x $10^{-5}$ | -ve | +ve | +ve | +ve | +ve | -ve | -ve | Gamma | +ve |
| S3W x $10^{-5}$ | +ve | -ve | +ve | +ve | -ve | -ve | +ve | Gamma | +ve |
| S3W x $10^{-3}$ | +ve | -ve | +ve | +ve | -ve | -ve | -ve | Beta | +ve |
| S2W x $10^{-3}$ | +ve | +ve | +ve | +ve | +ve | -ve | +ve | Beta | +ve |
| S2W x $10^{-5}$ | +ve | +ve | -ve | -ve | -ve | -ve | -ve | Beta | +ve |

KEY: S: Section, F: Floor, B: Body, W: Wall, Key+: Positive, Key - : Negative

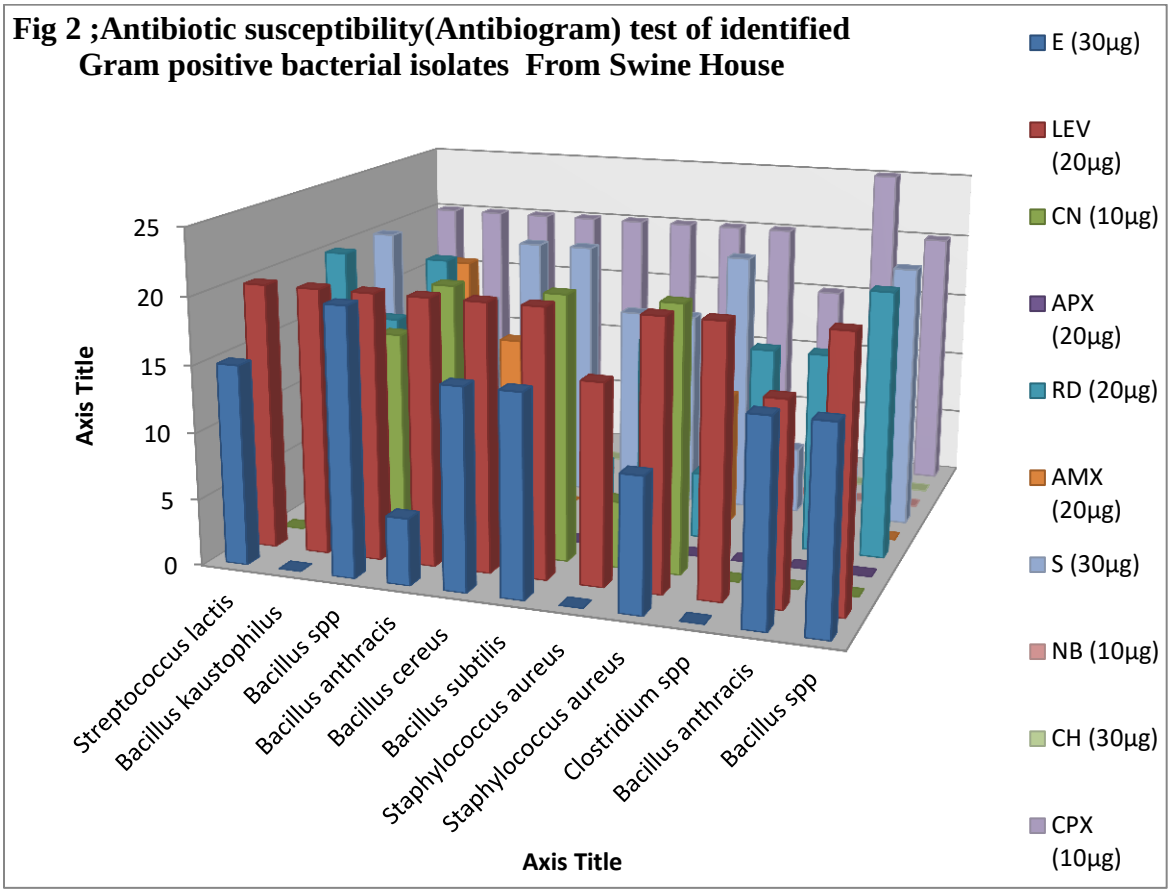
**Table 7**; Sugar fermentation Tests for bacteria identification of isolates From Floor, Body and Wall of Swine House

| Sample code(Swine House floor) | Lactose | Sucrose | Dextrose | $H_2S$ | Gas production |
|---|---|---|---|---|---|
| S2F x $10^{-3}$ | -ve | -ve | -ve | -ve | -ve |
| S3F x $10^{-3}$ | +ve | +ve | +ve | -ve | -ve |
| S3F x $10^{-5}$ | +ve | +ve | +ve | -ve | -ve |
| S2B x $10^{-3}$ | +ve | +ve | +ve | +ve | -ve |
| S3B x $10^{-3}$ | +ve | +ve | +ve | -ve | -ve |
| S3B x $10^{-5}$ | +ve | +ve | +ve | -ve | -ve |
| S3B x $10^{-5}$ | +ve | +ve | +ve | -ve | -ve |
| S3W x $10^{-5}$ | -ve | -ve | -ve | -ve | -ve |
| S3W x $10^{-3}$ | -ve | -ve | -ve | -ve | -ve |
| S2W x $10^{-3}$ | +ve | +ve | +ve | +ve | -ve |
| S2W x $10^{-5}$ | +ve | +ve | +ve | -ve | -ve |

Key: S: Section, F: Floor, B: Body, W: Wall, Key+: Positive, Key - : Negative

**Table 8** : Probable organisms From Floor, Body and Wall of Swine House

| Sample code(Swine House floor) | Organisms identified |
|---|---|
| S2F x $10^{-3}$ | *Mycobacterium tuberculosis* |
| S3F x $10^{-3}$ | *Bacillus spp* |
| S3F x $10^{-5}$ | *Bacillus spp* |
| S2B x $10^{-3}$ | *Bacillus anthracis* |
| S3B x $10^{-3}$ | *Bacillus cereus* |
| S3B x $10^{-5}$ | *Bacillus subtilis* |
| S3B x $10^{-5}$ | *Staphylococcus aureus* |
| S3W x $10^{-5}$ | *Staphylococcus aureus* |
| S3W x $10^{-3}$ | *Clostridium spp* |
| S2W x $10^{-3}$ | *Bacillus anthracis* |
| S2W x $10^{-5}$ | *Bacillus spp* |




KEY : S - Streptomycin, NB – Norflaxacin, CH- Chloramphenicol, CPX- Ciproflox, E- Erythromycin, LEV- Levofloxacin, CN- Gentamycin, APX- Ampiclox, RD- Rifampicin, AMX-Amoxil.

**Fig 2:** Shows the antibiotic susceptibility test of the identified Gram positive organisms. It was observed that *Streptococcus lactis*, *Bacillus anthracis* and *Bacillus cereus* has the highest zone of inhibition to streptomycin (20mm), while, *Bacillus kaustophilus* has the lowest zone of inhibition to streptomycin (0mm). *Bacillus anthracis, Bacillus subtilis* have the highest zone of inhibition to gentamycin(20mm), while *Bacillus kaustophilus* , *Streptococcus lactis, Bacillus cereus* have the lowest zone of inhibition to gentamycin(00mm). *Streptococcus lactis, Bacillus spp* and *Bacillus anthracis* has the highest zone of inhibition to rifampicin(20mm), while *Bacillus cereus* has the lowest zone of inhibition to rifampicin(00mm) and many other organisms shows high suspectibility to the antibiotics used.

**Fig 3**; Percentage inhibition ratio of Erythromycin against isolated organism from Swine House *Bacillus anthracis* 14%, *Staphylococcus aureus* 9%, *Bacillus subtilis* 14%, *Bacillus cereus* 14%, *Bacillus anthracis* 4%, *Bacillus spp* 18%, *Streptococcus lactis* 13%, *Bacillus spp* 14%.

**Fig 4**; Percentage inhibition ratio of 0.525λ and Bacillus subtilis have the lowest Levofloxacin against isolated organism from Swine House. Bacillus subtilis 10%, Staphylococcus aureus 7%, Staphylococcus aureus 10%, Clostridium spp 10%, Bacillus anthracis 7%, Bacillus spp 10%, Streptococcus lactis 9%, Bacillus kaustophilus 9%, Bacillus spp 9%, Bacillus anthracis 9%, Bacillus cereus 10%.

**Fig 5**; Percentage inhibition ratio of Gentamycin against isolated organism from Swine House. Staphylococcus aureus 25%, Staphylococcus aureus 6%, Bacillus subtilis 25%, Bacillus anthracis 24%, Bacillus spp 20%. Bacillus cereus 0%.

**Fig 6**; Percentage inhibition ratio of Rifampicin, against isolated organism from Swine House. Staphylococcus aureus 12%, Staphylococcus aureus 4%, Clostridium spp 12%, Bacillus anthracis 12%, Bacillus spp 15%, Streptococcus lactis 15%, Bacillus kaustophilus 11%, Bacillus spp 15%, Bacillus subtilis 4%.

**Fig 7**; Percentage inhibition ratio of *A*moxil against isolated organism from Swine House Staphylococcus aureus 24%, Bacillus anthracis 31%, Bacillus spp 45%.

**Fig 8**; Percentage inhibition ratio of Streptomycin against isolated organism from Swine House. Bacillus subtilis 9%, Bacillus cereus 13%, Staphylococcus aureus 9%, Staphylococcus aureus 12%, Clostridium spp 3%, Bacillus anthracis 3%, Bacillus spp 12%, Streptococcus lactis 13%, Bacillus kaustophilus 4%, Bacillus spp 9%, Bacillus anthracis 13%, Bacillus cereus 13%.

**Fig 9**; Percentage inhibition ratio of Ciproflox against isolated organism from Swine House. Staphylococcus aureus 9%, Clostridium spp 7%, Bacillus anthracis 12%, Bacillus spp 9%, Streptococcus lactis 9%, Bacillus kaustophilus 9%, Bacillus spp 9%, Bacillus anthracis 9%. Bacillus cereus 9%.

**Fig 10**.: Shows the growth dynamic of bacteria isolates using ultraviolet spectrophotometer with the wavelength 480λ. It was observed that at 0hour, Bacillus spp has the highest growth rate of growth rate of 0.001λ. At 84th hour, bacillus spp has the lowest death rate of 0.307 λ and Bacillus cereus have the highest death rate of 0.227λ.

**Fig 11** : Shows the growth dynamic and killing time of bacteria isolates and addition of ciprofloxacin antibiotic at 24th hour using ultraviolet spectrophotometer. In this table, it was observed that at 0 hour, Bacillus subtilis has the highest growth rate of 0.251λ and Bacillus cereus have the lowest growth rate of 0.019λ. At the 84th hour, Bacillus kaustophilus has the lowest death rate of 0.152λ and Bacillus subtilis have the highest death rate of 0.097.

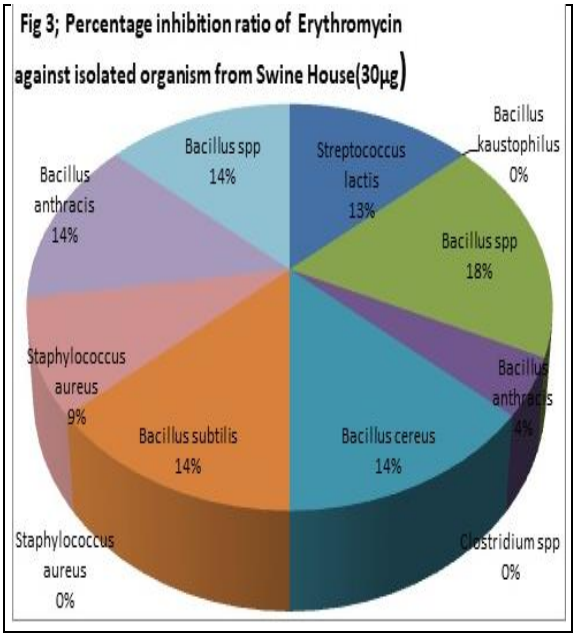


Fig 3; Percentage inhibition ratio of Erythromycin against isolated organism from Swine House(30µg)
Bacillus spp 14%
Streptococcus lactis 13%
Bacillus kaustophilus 0%
Bacillus anthracis 14%
Bacillus spp 18%
Staphylococcus aureus 9%
Bacillus anthracis 4%
Bacillus subtilis 14%
Bacillus cereus 14%
Staphylococcus aureus 0%
Clostridium spp 0%

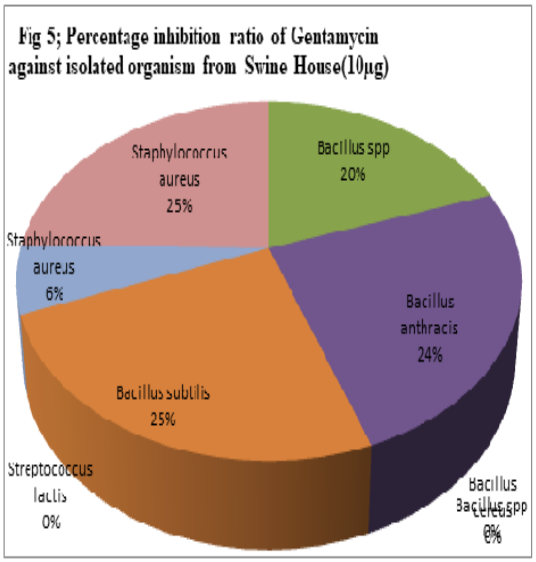


Fig 5; Percentage inhibition ratio of Gentamycin against isolated organism from Swine House(10µg)
Staphylococcus aureus 25%
Bacillus spp 20%
Staphylococcus aureus 6%
Bacillus anthracis 24%
Bacillus subtilis 25%
Streptococcus lactis 0%

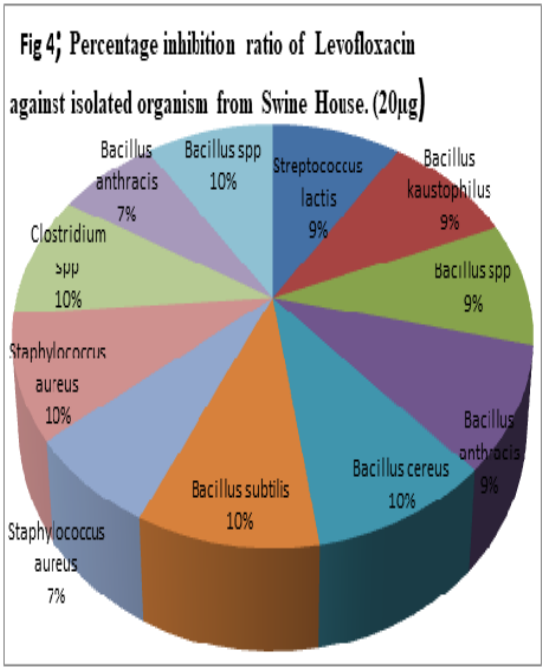


Fig 4; Percentage inhibition ratio of Levofloxacin against isolated organism from Swine House. (20µg)
Bacillus anthracis 7%
Bacillus spp 10%
Streptococcus lactis 9%
Bacillus kaustophilus 9%
Clostridium spp 10%
Bacillus spp 9%
Staphylococcus aureus 10%
Bacillus anthracis 9%
Bacillus subtilis 10%
Bacillus cereus 10%
Staphylococcus aureus 7%

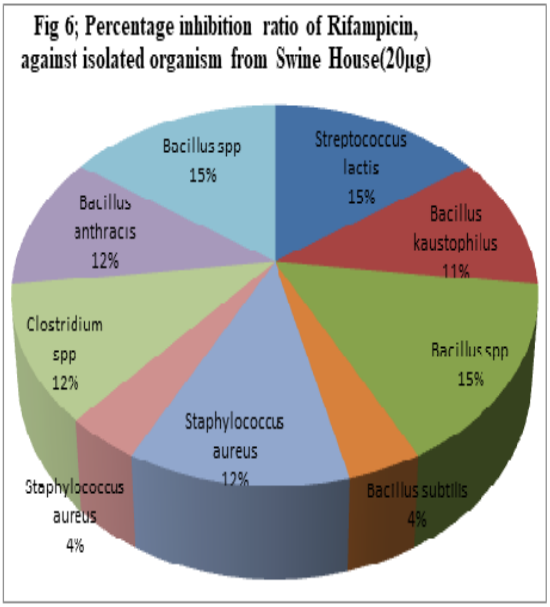


Fig 6; Percentage inhibition ratio of Rifampicin, against isolated organism from Swine House(20µg)
Bacillus spp 15%
Streptococcus lactis 15%
Bacillus anthracis 12%
Bacillus kaustophilus 11%
Clostridium spp 12%
Bacillus spp 15%
Staphylococcus aureus 12%
Staphylococcus aureus 4%
Bacillus subtilis 4%

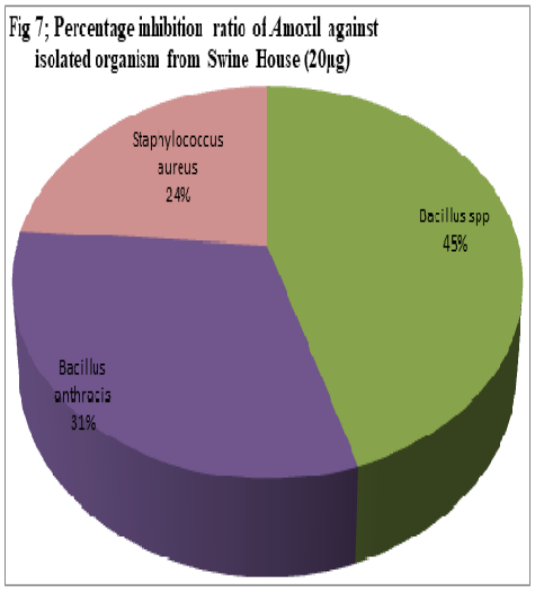


Fig 7; Percentage inhibition ratio of Amoxil against isolated organism from Swine House (20µg)
Staphylococcus aureus 24%
Bacillus spp 45%
Bacillus anthracis 31%

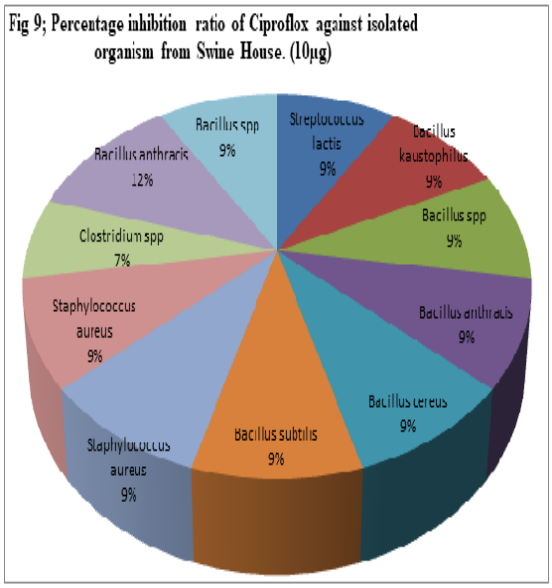


Fig 9; Percentage inhibition ratio of Ciproflox against isolated organism from Swine House. (10µg)
Bacillus spp 9%
Streptococcus lactis 9%
Bacillus kaustophilus 9%
Bacillus anthracis 12%
Bacillus spp 9%
Clostridium spp 7%
Staphylococcus aureus 9%
Bacillus anthracis 9%
Bacillus cereus 9%
Bacillus subtilis 9%
Staphylococcus aureus 9%

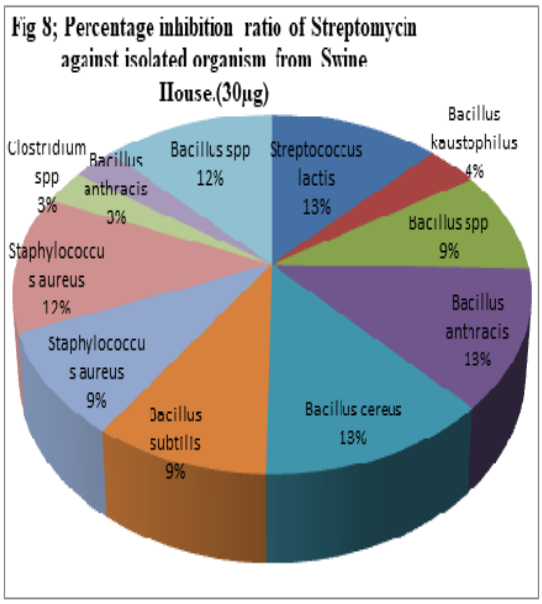


Fig 8; Percentage inhibition ratio of Streptomycin against isolated organism from Swine House.(30µg)
Clostridium spp 3%
Bacillus anthracis 3%
Bacillus spp 12%
Streptococcus lactis 13%
Bacillus kaustophilus 4%
Bacillus spp 9%
Staphylococcus aureus 12%
Staphylococcus aureus 9%
Bacillus anthracis 13%
Bacillus subtilis 9%
Bacillus cereus 13%

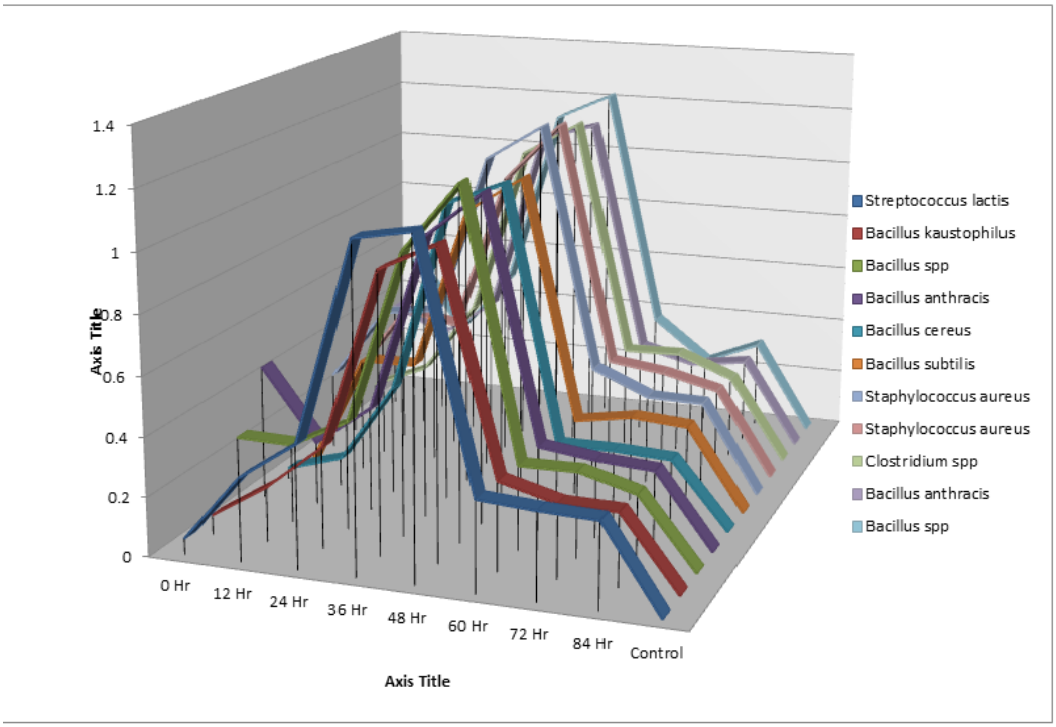
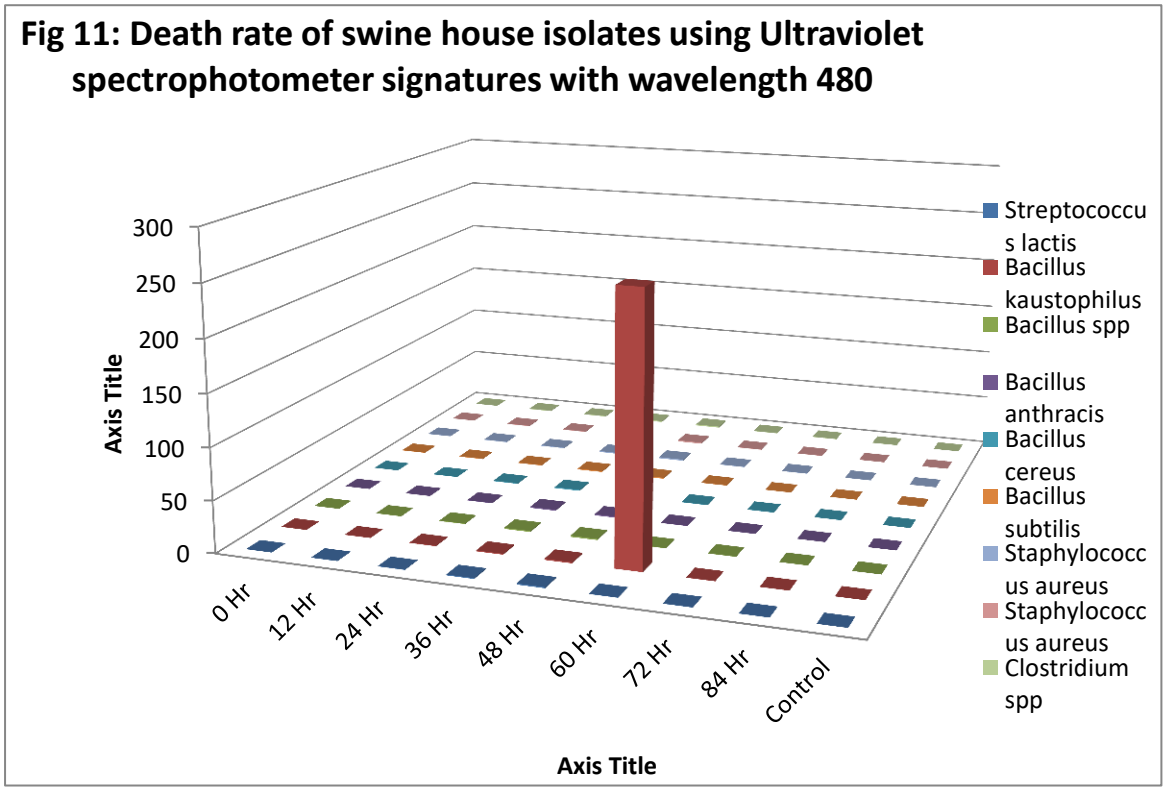



**Fig 10: Growth dynamic of swine house isolate using Ultraviolet spectrophotometer signatures with wavelength 480$\lambda$**



## CONFIRMATORY MOLECULAR CHARACTERIZATION OF BACTERIA ISOLATES FROM SWINE HOUSE

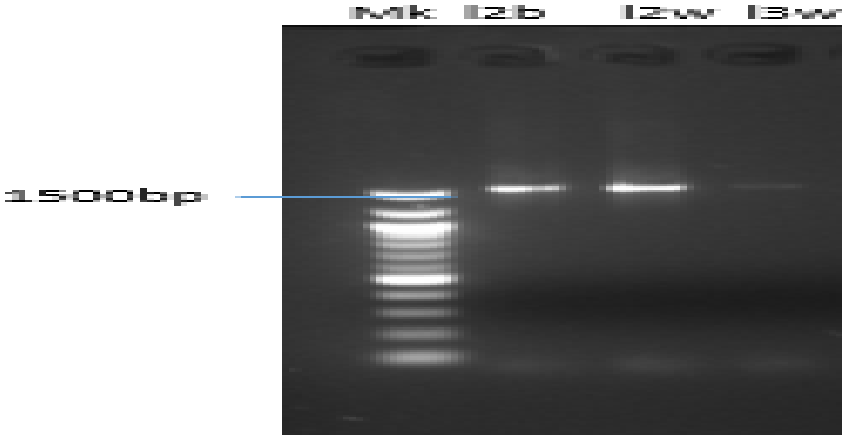



**Plate 1**: Agarose gel electrophoresis showing the positive amplification of the 16S rRNA region of bacteria isolated from body of swine and wall of where they are reared.

**Table 9**: NCBI Blast Result Showing Confirmatory Identification of Swine House Bacterial Isolates.

| Select for downloading or viewing reports | Scientific Name | Max Score | Total Score | Query Cover | E value | Per. Identity | Accession |
|---|---|---|---|---|---|---|---|
| L2 wall | Shigella flexneri | 2588 | 18003 | 99% | 0 | 99.93% | OK058818 |
| L3 wall | Enterococcus faecalis | 2513 | 2513 | 99% | 0 | 99.86% | OK058819 |
| L2 body | Enterococcus faecalis | 2614 | 10457 | 99% | 0 | 99.86% | OK058820 |

>OK058818 Shigella flexneri strain L2W

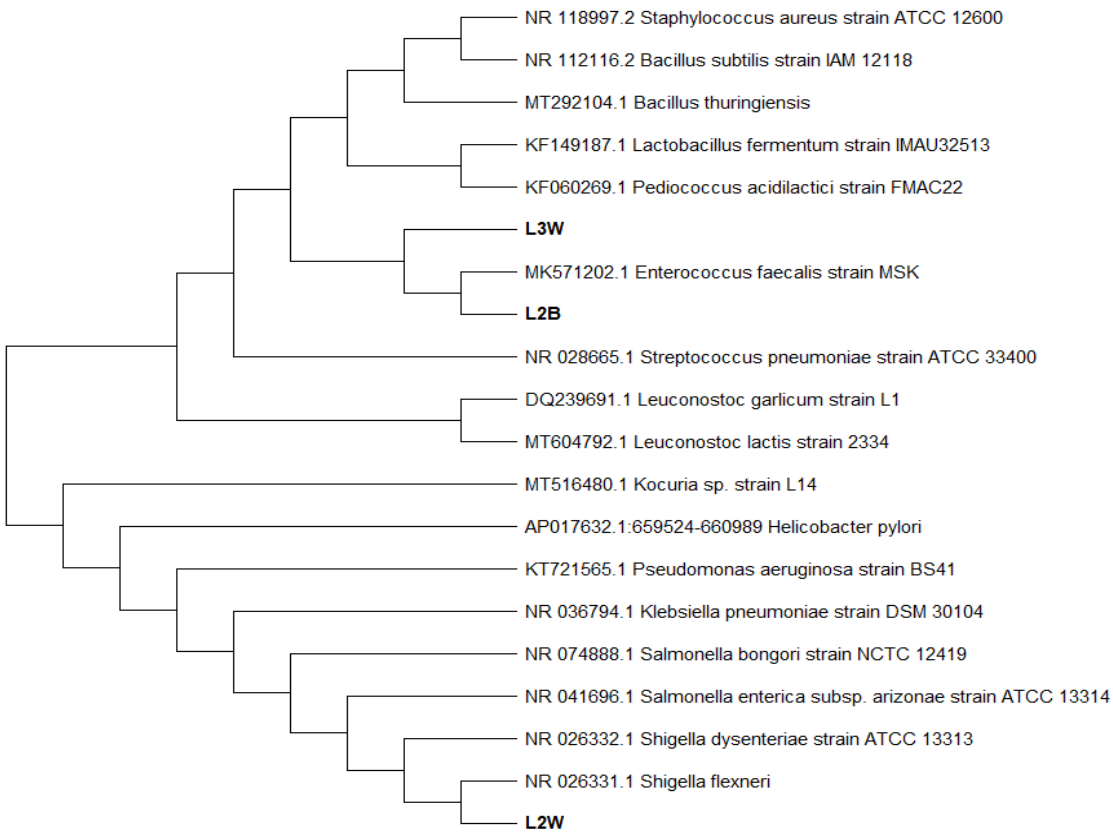



**Fig 12:** Molecular assay of characterization of *Shigella flexneri , Enterococcus faecalis and Enterococcus faecalis*

# DISCUSSION

The objective of the study was to access microbial load and microorganism found in swine house depending on the sample site and to compare between the conventional and molecular methods of characterization of swine house isolates. The antimicrobial spectrum, growth/ killing kinetics of the isolates using Ultraviolet spectrophotometer signatures were also evaluated. Morphological characteristics of isolates was studied base on surface, colour, elevation, edge and mode of spread, findings show that surface of the isolates either appear as dry, wet, smooth or rough. they also appear as either milky or creamy in colour. The angle of elevation is either raised or low while isolates has it mode of spread as moderate or swarm. The conventional method of characterization isolates using polymerized chain reaction (PCR) were employed during the course of this research work Biochemical test for identifying microbe was dually observed for the isolated bacteria.

In order to further identify the organism isolated according to the "Bergey's Manual of the Determinative Bacteriology". the isolated organism were identified to the Genus level. Findings revealed that all isolated bacterial were Gram positive and appears to have short rods. Further biochemical test were performed Result shows that all isolates were non motile Most isolates were indole positive and also has the ability of utilizing one or two sugars as well as production of gas . Further test such as catalase, oxidase mannitol, maltose, glucose, M.R ,V.P test were all carried out and organism isolated include; *Bacillus spp, Bacillus anthracis, Bacillus cereus, Bacillus subtilis, Staphylococcus aureus, Clostridium spp,* with the conventional methods. While *Shigella flexneri, Enterococcus faecalis and Enterococcus faecalis* with the PCR confirmatory method, this agrees to the work of Lei *et al.* (31,32,33) ,

Bacteria isolated was tested against various antibiotics such as Streptomycin, Norfloxacin, Chloramphenicol, Ciproflox, Erythromycin, Levofloxacin, Gentamycin, Ampiclox, Rifampicin, and Amoxil. Result shows that Levofloxacin, Erythromycin, Ciproflox and Gentamycin shows susceptibility rate on all tested bacteria while all tested organisms were resistant to Norfloxacin, Chloramphenicol and Amoxil. Other tested antibiotics has intermediate, resistant or are susceptible to one or two isolates. All antibiotics used for this research has a frequency inhibiton potentials on the isolates. Erythromycin shows percentage inhibition of *Bacillus anthracis* 14%, *Staphylococcus aureus* 9%, *Bacillus subtilis* 14%, *Bacillus cereus* 14%, *Bacillus anthracis* 4%, *Bacillus spp* 18%, *Streptococcus lactis* 13%, *Bacillus spp* 14%, Levofloxacin shows percentage inhibition of Bacillus subtilis 10%, Staphylococcus aureus 7%, Staphylococcus aureus 10%, Clostridium spp 10%, Bacillus anthracis 7%, Bacillus spp 10%, *Streptococcus lactis* 9% ,*Bacillus kaustophilus* 9%, Bacillus spp 9%, *Bacillus anthracis* 9%, Bacillus cereus 10%, Gentamycin shows percentage inhibition of *staphylococcus aureus* 25%, *Staphylococcus aureus* 6%, Bacillus subtilis 25%, *Bacillus anthracis* 24%, *Bacillus* spp 20%. *Bacillus cereus* 0%. Rifampicin shows. *Staphylococcus aureus* 12%,, *Staphylococcus aureus* 4%, *Clostridium* spp 12%, *Bacillus anthracis* 12%, *Bacillus* spp 15%, *Streptococcus lactis* 15%, *Bacillus kaustophilus* 11%, Bacillus spp 15%, *Bacillus subtilis* 4%, *A*moxil shows percentage inhibition of Staphylococcus aureus 24%, *Bacillus anthracis* 31%, *Bacillus* spp 45% Streptomycin, *Bacillus subtilis* 9%, Bacillus cereus 13%, Staphylococcus aureus 9%, Staphylococcus aureus 12%, Clostridium spp 3%, Bacillus *anthracis 3*%, *Bacillus* spp 12%, *Streptococcus lactis* 13%, *Bacillus kaustophilus* 4%, *Bacillus* spp 9%, *Bacillus anthracis* 13%, *Bacillus cereus* 13% and Ciproflox shows percentage inhibition of against *Staphylococcus aureus* 9%, C*lostridium* spp 7%, *Bacillus anthracis* 12%, Bacillus spp 9%, *Streptococcus lactis* 9%, *Bacillus kaustophilus* 9%, *Bacillus spp* 9%, *Bacillus anthracis* 9%. *Bacillus cereus* 9%.

*Shigella flexneri and Enterococcus faecalis* were the two organism isolated abd conferned by PCR is a pathogenic organism , it is advisable to wash the swine meat properly before eating and farmers that specializes in the production of pigs should take adequate precaution in the farm to avoid been contaminated with this pathogenic organism. Shigella flexneri is a species of Gram-negative bacteria in the genus Shigella that can cause diarrhea in humans. Several different serogroups of Shigella are described; S. flexneri belongs to group B. S. flexneri infections can usually be treated with antibiotics, although some strains have become resistant. *Shigella* species invade the colonic and rectal epithelium of primates and humans, causing the acute mucosal inflammation characteristic of shigellosis (34). Infection is usually confined to the superficial layer of the colonic mucosa, where severe tissue damage leads to abscesses and ulceration. Destruction of the epithelial layer leads to the clinical symptoms of watery diarrhoea, severe abdominal pain and cramping, eventuating in the bloody mucoid stool characteristic of bacillary dysentery. In the absence of effective treatments, shigellosis patients may develop secondary complications such as septicaemia, pneumonia and haemolytic uremic syndrome (35)

Enterococcus faecalis is formerly classified as part of the group D Streptococcus system – is a Gram-positive, commensal bacterium inhabiting the gastrointestinal tracts of humans. Like other species in the genus Enterococcus, E. faecalis is found in healthy humans and can be used as a probiotic. *E.faecalis* normally lives harmlessly in your intestines. However, if it spreads to other parts of your body it can cause a more serious infection. The bacteria can get into your blood, urine, or a wound during surgery. From there, it can spread to different sites causing more serious infections, including sepsis, endocarditis, and meningitis. Enterococcus faecalis and E. faecium cause a variety of infections, including endocarditis, urinary tract infections, prostatitis, intra-abdominal infection, cellulitis, and wound infection as well as concurrent bacteremia. Enterococci are part of the normal intestinal flora(36). Growth dynamic of bacteria isolates using

ultraviolet spectrophotometer signatures shows that the highest growth of 0.132λ was observed in *Enterobacter spp* while the least growth was observed in *Shigella flexneri* ,at 0.021λ. likewise, at 60th hour, *Bacillus spp* has the lowest death rate of 0.140λ and *Enterobacter spp* have the highest death rate of -0.057λ growth dynamic and killing time of bacteria isolates and the addition of ciprofloxacin antibiotic at 24th hour using ultraviolet spectrophotometer. At 0 hour, highest growth rate of 0.181λ was observed in *Bacillus cereus,* while the least growth rate was observed in *Enterobacter* spp at 0.135λ. At the 60th hour, *Bacillus spp* has the lowest death rate of -0.080λ and *Enterobacter spp* have the highest death rate of -0.050λ.. *Bacillus spp become resistant at* 60th hour (21). It was observed that, it a lot accurate to characterized clinical and environmental isolates with the molecular method, compared with normal conventional method. The molecular methods gives over 99% purity to the sub species level compared with the conventional method. But it is imperative to know that the conventional method can be used as the preliminary method and conventional methods may be used as the confirmatory method, this is demonstrated during the course of this research work, and the result is tremendous. Undercooked pork can transmit *Shigella flexneri, Enterococcus faecalis and* Yersinia bacteria, causing short-term illness and raising the risk of reactive arthritis, chronic joint conditions, Graves' disease and other complications. Even if there is need to consume pork meat (37). It can be deduced from finding of this research work, that there is absolute and inherent danger pig house (37). Farmed and handler needed to be extra care in keeping this as pet and farm animal.

## Conclusion

Molecular based methods are complementary to conventional methods and are revolutionizing microbial diversity and taxonomy research as well as applied fields. The characterization of tested organisms. Results of the conventional characterization indicate the preliminary identity of the isolated organism under the genus *Bacillus, Enterococcus, Clostridium, Staphylococcus and Shigella.* Molecular characterization based on MEGA 6a rDNA sequencing and NCBI BLAST were *Enterococcus faecalis and shigella flexneri* strain. it will be appropriate to consider more time in cooking of pig meat before consumption. , to reduce this scourge of this pathogenic microorganisms. pig farmers should also consider the use of different arrays of antibiotics in disinfecting the swine house regularly , to reduce the microbial load, as stated in the finding of this research work. To avoid parasitic infection, always cook pork thoroughly. Check the temperature with a meat thermometer to ensure the meat has become hot enough to kill parasites and bacteria before serving.

## References

1. Buczynska, A. and Szadkowska-Stańczyk, I. (2010). Occupational hygiene and health hazards related to concentrated animal feeding operations. *Journal of Health.* 61: 323–331.

2. Jurek, A., Szejniuk, B., Wit, B. and Michalska, M. (2006). Microbiological air contamination during piglet rearing. *Journal of applied Science.*61: 33–38.

3. Kluczek, 2002). Kluczek S. (2002). Population of microorganisms in the air of piggery in the course of breeding of sows with piglets and weaners. *Journal of Animal science.* 50: 33-48.

4. Nowak, B. Von Müffling, T., Chaunchom, S. and Hartung, J. (2007). *Salmonella* contamination in pigs at slaughter and on the farm. *International Journal of Food Microbiology.* 115: 259–267.

5. Letellier, A., Messier, S., Pare, J., Menard, J. and Qessy, S. (2012). Distribution of *Salmonella* in swine herds in Quebec. *Clinical Microbiology.* 67: 299-306.
6. Tombarkiewicz, B., Niedziółka, J., Migdał, W., Lis, M., Pawlak, K., Podgórni, Z, and Lubkiewicz, M. (2000). The attempt to evaluate a range of microbiological contamination of the environment of a pig farm. *Journal of Biological Science.*46: 37–42.

7. Kiers, J.L., Nout, M.J.R., Rombouts, F.M., Nabuurs, M.J.A., VanDer,F. and Moulen, J. (2007). A high molecular weight soluble fraction of tempeh protects against fluid losses in *Escherichia coli* infected piglet small intestine. *Journal of Nutrition.* 98: 320-325.

8. Wernli, D., Emonet, S., Schrenzel, J. and Harbarth, S. (2011). Evaluation of eight cases of confirmed *Bordetella bronchiseptica* infection and colonization over a 15-year period. Clin Microbiol Infect.17: 201-203.

9. Habteselassie, M., Bischoff, M., Blume, E., Applegate, B., Reuhs, B., Brouder, S. and Turco, R.F. (2008). Environmental controls on the fate of *Escherichiacoli* in soil. *Water, Air and Soil Pollution.*190: 143–155.

10. Boes, J., Alban, L., Bagger, J., Møgelmose, V., Baggesen, D.L. and Olsen, J.E. (2005). Survival of *Escherichia coli* and *Salmonella typhimurium*in slurry applied to clay soil on a Danish swine farm. *Journal of Medical Microbiology.* 69: 213–228.

11. Takahashi, E., Furkawa, C., Yamoto, A., Okada, K., Okada, M., Watanabe, S., Furuya, S.U. and Kimoto, H. (2000). Seasonal changes of bacterial flora in an activated sludge system for swine wastewater. *Journal of Zoo-technical Science.* 71: 362–369.

12. Tymczyna, L., Trawińska, B. and Saba, L. (2010). Microbiological pollution of some environmental components around the pig farm. *Journal of Animal Science.* 26: 133–142.

13. Hoelzer K., Switt A.I.M., Wiedmann M. (2011). Animal contact as a source of human nontyphoidal salmonellosis. *Journal of applied Science.*42: 34–65.

14. Williamson, G. and Paye, W. J. A. (2011). An introduction to Animal Husbandry in the Tropics. *Journal of Biological science.*7:782-791.

15. LeSciellour, M., Labussière, E., Zemb, O., Renaudeau, D. (2018). Eect of dietary fiber content on nutrient digestibility and fecal microbiota composition in growing-finishing pigs. *Journal of Clinical Microbiology.*13: 1412-1420.

16. Vahjen, W., Pieper, R. and Zentek, J. (2010). Bar-coded pyrosequencing of 16S rRNA gene amplicons reveals changes in ileac porcine bacterial communities due to high dietary zinc intake. *Applied Environmental Microbiology.* 76:6689–6691.

17. Leser, T.D., Amenuvor, J.Z., Jensen, T.K., Lindecrona, R.H., Boye, M. and Moller, K. (2002). Culture-independent analysis of gut bacteria: The pig gastrointestinal tract microbiota revisited. *Applied Environmental Microbiology.*68,673–690.

18. Tan, Z., Wang, Y., Yang, T., Ao, H., Chen, S., Xing, K., Zhang, F., Zhao, X., Liu, J. and Wang, C. (2018). Differences in gut microbiota composition in finishing Landrace pigs with low and high feed conversion ratios. *Journal of Clinical Microbiology.* 111: 1673–1685.

19. Isaacson, R. and Hyeun, B.K. (2012). The intestinal microbiome of the pig. *Animal Health Resource Review.* 3:100–109.

20. Osuntokun OT, Azuh VO, Adejoro BF, Akele EO. (2021a)Antimicrobial Spectrum, Growth/Killing Kinetics, Conventional/Molecular Assay of Characterizing Non-Leguminous Endophytic Bacteria and Fungi from *Helianthus annuus*, *Carica papaya* and *Lycoperesicum solanum*. J Biomed Res Environ Sci. 2021 Oct 30; 2(10): 1018-1034.

21. Osuntokun OT, Balogun JO, Akele EO,Adedayo SA, Bello OA(2021b). Aframomum melegueta (Roscoe) K. Schum) Ethanolic Seed Extract against Asymptomatic Bacteriuria Isolates from Pregnant Women Attending Antenatal Clinic in Ondo State, Nigeria. International Journal of Pathogen Research, 2021;8(2):12-43.

22. Olutiola, P.O., Famurewa, O. and Sonntang, H.G. (2000). An Introduction to microbiology, practical Approach. *Tertiary Text Book Series.* pp. 45.

23. Bergey DH, Holt JG, Noel RK. Bergey's Manual of Systematic Bacteriology, 19th Ed.1994. https://tinyurl.com/3xz4k3ec.

24. Cappuccino, J.G. and Sherman, N. (2002). Microbiology: A laboratory manual. Pearson Education Incorporated.

25. Baker CN, Thornsberg CH (1983). Inoculum standardization in antimicrobial susceptibility tests: Evaluation of overnight age culture. J. Chin. Microbial. (17): 140-457.

26. MacFaddin, J.F. (2000). Biochemical Tests for Identification of Medical Bacteria. 3rd Edition,

27. Kato H, Furuhashi T, Tanaka M, Katsu Y, Watanabe H, Ohta Y, Iguchi T. 2006. Effects of bisphenol A given neonatally on reproductive functions of male rats. Reprod Toxicol 22(1):20-29.

28. Fawole MO, Oso BA. Laboratory manual of Microbiology. Revised Edition spectrum books Ltd. Ibadan. 2001;127.

29. Clinical and Laboratory Standards Institute; Wayne PA. Methods for dilution antimicrobial susceptibility testing for bacteria that grew aerobically. 2009;M7-A10.

30. Osuntokun, O. T., Bankole, O. M., Abike, T. O., Joy, O. E., & Adenike, A. F. (2020a). Systematic Comparative Study of Selected Antibiotics and Sulphur/ Medicinal Plant Mediated Nano-particles against Non-Leguminous Endophytic Bacteria and Clinical Isolates. *Journal of Applied Life Sciences International*, *23*(10), 43-56.

31. Osuntokun O.T, Ibukun A.F, Yusuf-Babatunde A.M, Abiodun S (2019) Pre/Post-Plasmid Profile Analysis, Killing- Kinetics and Secondary Metabolites Screening of *Adenopus breviflorus (Benth)* Fruit Extract Against Multiple Drug Resistant Isolates Using *Staphylococcus aureus (MDRSA)* as a Case Study. Journal of Advanced Research in Biotechnology, J Adv Res Biotech Vol 4(1):1-17.

32. Osuntokun O.T & Mary, T. T. (2020b).Comparative Study of Ethanolic Wild African Nutmeg (Pycnanthus angolensis (Welw.) Stem Bark Extract Potentials and Selected Conventional Tooth paste against Hidden Resident Mouth Cavity Microfora. *Asian Journal of Biochemistry, Genetics and Molecular Biology*, *6*(2), 10-36. https: //doi.org/10. 9734/ajbgmb/2020/v6i230147.

33. Lei X, Wang ET, Chen WF, Sui XH, Chen WX. Diverse bacteria isolated from roonodules of wild Vicia species grown in temperate region of China. Arch Microbiol.2008 Dec;190(6):657-71. doi: 10.1007/s00203-008-0418-y. Epub 2008 Aug 15. PMID: 18704366.

34. Bennish M.L. ojtyniak B.J.(1991) Mortality due to shigellosis: community and hospital data Rev. Infect. Dis 13 S245–S251.

35. Goldberg, Marcia B. (2001). "Actin-Based Motility of Intracellular Microbial Pathogens". Microbiology and Molecular Biology Reviews. 65 (4): 595–626. doi:10. 1128/ MMBR.65.4.595-626.2001.

36. Anthee, Suresh; Paudel, Atmika; Hamamoto, Hiroshi; Ogasawara, Akihiko Ano; Iwasa, Toshihiro; Blom, Jochen; Sekimizu, Kazuhisa (2021). "Complete genome sequence and comparative genomic analysis of Enterococcus faecalis EF-2001, a probiotic bacterium". *Genomics*. 113 (3):1534–1542.

37. Dibi E.A.B., Zita E.B.N., Djedjro C.A., Tano K., Assidjo E.N. Risques microbiens lies a la consommation de la viande bovine braisee « *Choukouya* » en Côte d'Ivoire. Int. J. Innov. Appl.

Stud. 2017;19(3):496–507. ISSN 2028-9324.

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