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First Case Report Of *Paenibacillus* sp From Pig Farms In Ogun State, Nigeria.

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ABSTRACT

Pig faecal samples (138) samples obtained from two piggery (Oke-Aro and Otta farms) in Ogun State were analyzed for the presence of Paenibacillus sp. using standard Microbiological methods. Thirty five 35(25%) isolates were positive with 23(34.8%) in Oke-Aro farms and 12(16.7%) in Otta farms. The distribution rates among different pig categories in each farm included Boars 1(9.1%), Sows 2(13.3%), Growers/Guilts 4(22.3%) and Weaners/suckers 5(17.9%) for Otta farms and Boars 3(37.5%), Sows 5(33.3%), Growers/Guilts 6(22.2%) and Weaners/Suckers 9(39.1%) for Oke-Aro farm. Oke Aro farm recorded a prevalence of 34.8% while Otta farm had a prevalence rate 16.7%. DNA sequencing of isolates from each farm using 16sRNA gene amplification identified *Paenibacillus konsidensis*, *Paenibacillus massiliensis, paenibacillus timonensis, Paenibacilus terrae in* Oke-Aro farm, and *Paenibacillus amyloticus, Paenibacillus polymyxa, Fontibacillus phaseoli* in Otta farm with a range of 95-99% identity.

Keywords: Paenibacillus species, Piggery, Molecular Characterization, 16sRNA, Nigeria

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INTRODUCTION

Bacteria belonging to the genus *Paenibacillus* have been isolated from a variety of environments, with many of the species being relevant to humans, animals, plants, and the environment. The majority of them are found in soil, often associated with plants roots: these rhizobacteria promote plant growth and can be exploited for use in agriculture (Grady *et al*, 2016; Padda *et al*., 2016a and b). Many species of *Paenibacillus* produce antimicrobial compounds that are useful in medicine or as pesticides, and many yield enzymes that could be utilized for bioremediation including xylanases, or to produce valuable chemicals (Lee *et al.,* 2012; Cochrane and Vederas, 2016). Previous studies have reported the isolation of different strains of this organism in patients undergoing haemodialysis, prosthetic joint infection, bacteremia in cerebral infection, Carcinoma intestinal nephropathy and leukemia (Ko *et al*., 2008, and Ouyang *et al.,* 2008). In accordance with their diverse characteristics, members of *Paenibacillus* have been discovered in disparate habitats, from Polar Regions to the tropics, and from aquatic environments to the driest of deserts. Species of *Paenibacillus* were originally included in the genus *Bacillus*, which historically was defined based on morphological characteristics in common with the type species *Bacillus subtilis*, isolated in 1872. Any bacterium was classified as *Bacillus* if it was rod-shaped, aerobic or facultatively anaerobic, and could form endospores allowing it to remain dormant in inhospitable conditions. However, these characteristics are actually very ancient and not suitable for grouping species into a single genus. A more accurate representation of phylogenetic relationships among these bacteria was attained in 1991, when 16S rRNA gene sequences were determined for standard strains of 51 species then defined as *Bacillus* (Ash *et al*., 1991). Using this technique, several species were also reclassified into the genus *Paenibacillus*, including *Clostridium durum*, *Bacillus alginolyticus*, *Bacillus chondroitinus*, *Bacillus curdlanolyticus*, *Bacillus glucanolyticus*, *Bacillus kobensis*, and *Bacillus thiaminolyticus*In 1997, a proposed emendation described *Paenibacillus* as having 16S rDNA sequences with more than 89.6% similarity, being motile by means of peritrichous flagella (projecting in all directions), and being non-pigmented on nutrient agar, among other characteristics. Species of this genus can be gram positive, gram negative, or gram variable, in addition to sharing the basal characteristics ascribed to *Bacillus* (Shida *et al*., 1997).

MATERIALS AND METHODS

Study Site And Design

This study was carried out in two locations Otta and Oke-Aro pig farms in Ogun states of Nigeria. This state is selected because of its relatively high livestock activities, it has sizeable expanse of arable land and rich fertile soils that are good for the cultivation of a wide variety of food crops and animal production. Generally, livestock activities in the area are on the increase and there is a high dependence on livestock as a source of employment, revenue and milk and meat production. There are large-scale livestock production industries particularly of cattle, sheep and goats in these areas, mainly in semi-intensive farming systems in which animals are taken out to graze and then returned to their pens later in the evening, besides other small intensive and semi-intensive livestock farming operations that characterize most households. In addition, there is unregulated access to veterinary drugs; a farmer could decide to purchase and administer drugs without veterinary prescription and supervision.

Sample Size

Using a single proportion formula, the sample size is calculated thus:

$$n = Z^2pq / d^2$$

Where: n = desired sample size, when population is more than 10,000
Z = Critical value at 95% or 1.96 confidence interval
p = Prevalence or population with the desired attribute pegged at 25% i.e 0.25
q = 1 – p
d = degree of accuracy usually set at 0.05

Hence, Z = 1.96, p = 0.25, q = 0.75, d = 0.05

$n = 1.96^2 \times 0.25 \times 0.75 / 0.05^2$ $n = 0.7203 / 0.0025$ $n = 288.12$ $n \approx 288$

Collection Of Stool Sample

A total of 138 pig faecal samples (72 from Otta farms and 66 from Oke-Aro farms) were collected between January and February, 2017 in sterile universal stool containers. The samples were transported to microbiology laboratory of university of Lagos, within 2hrs for processing. Samples were not collected in any preservative. Prior to discarding, specimen were sterilized by autoclaving.

Culture And Isolation

Streak plate method was used to isolate anaerobes from pig stool sample. The media used in this study included, Columbia agar with 5% defibrinated sheep's blood (Oxoid, UK), non-selective media used were Fastidious Anaerobe Agar (FAA, Lab M), cooked meat broth (Oxoid, UK) and Mueller Hinton Agar (SRL, India). All samples were subjected to alcohol shock treatment by homogenizing the specimen with an equal volume of 95% ethanol at room temperature for 1 hr to destroy all vegetative cells leaving only spores. They were subsequently dispensed into bottles containing Robertson's cooked meat Broth (CMB) for enrichment and the lids tightly covered.

The mixtures was then allowed to settle and a loopful of the deposit was aseptically streaked on Columbia agar for 48 hours, colonies after 48hrs of incubation were visible with the following characteristics, shiny, smooth with highly irregular forms, Colonies were then sub-cultured onto Fastidious Anaerobe Agar (FAA, Lab M) to obtain pure colonies in N_2(80%), H_2(10%) and CO_2 (10%), plates were incubated anaerobically at 37°C for 48hrs in an Anaerobic Jar (Biomerieux, France) in N_2(80%), H_2(10%) and CO_2 (10%). The surfaces of the plates were dried in the incubator at 30°C before use. Inoculated plates were incubated immediately under anaerobic conditions in an anaerobic jar (Biomerieux, France) in N_2(80%), H_2(10%) and CO_2 (10%) with an indicator (the rezusurin strip) to check anaerobiosis (disposable anaerobic indicator).

Media was reduced prior to inoculation by placing under anaerobic conditions for 24 h prior to use through the use of GasPak anaerobic systems. All plates showing no growth were further re-incubated for another 24 hours before being discarded. Control culture strain of *Pseudomonas aeruginosa* were plated alongside, this was to check anaerobiosis, because anaerobes grow well in anaerobic condition while *P. aeruginosa* which is a strict aerobe will not grow in the anaerobic jar. Pure cultures were then inoculated and stored into prepared cooked meat broth for morphological and biochemical characterization.

Identification of Isolates

Isolates were identified morphologically using Gram stain reagent and endospore staining in other to identify the spore formers.

Gram Staining

A smear of each isolates was prepared on a glass slide and heat fixed by passing briskly back and forth over a flame. The smear was then flooded with crystal violet and left for 30 seconds after which it was rinsed with water. The smear was covered with Gram iodine for 1minute (mordant) after which the excess iodine was rinsed off, the smear was decolorized with 70% alcohol for 10-30 seconds and rinsed off with tap water. Safranin was then added and left for 30 seconds and rinsed off with tap water. The slides were then allowed to air dry and then examined under the microscope using the oil immersion lens (×100). Samples that stained negative were discarded and the Gram positive samples where further stained to identify spore formers (endospore staining).

Endospore Staining

A smear of the sample was made on a clean slide, air-dried and heat-fixed, a small piece of blotting paper (absorbent paper) was placed over the smear, the slide was then placed (smear side up) on a wire guaze on a ring stand, it was then droped on a staining rack that has been placed over a boiling water bath until steam is coming up from the water, after which the heat was turned down so that the water is barely boiling. As the paper begins to dry the smear was then flooded with the primary dye, malachite green, and left for 5 minutes, the paper towel (blotting paper) was removed and allowed to cool to room temperature for 2 minutes. The slide

was then washed thoroughly with tap water (to wash the malachite green from both sides of the slides) and moved from the boiling water to the regular stain tray were the smear was flooded with the counterstain dye, safrinin, and left for 1 minute after which it was properly washed with tap water and blot dry with bibulous paper. Spores will be a light green while Vegetative cell walls will pick up the counterstain safranin.

Motility Test

Paenibacillus species are mostly motile. This test was carried out by the hanging drop technique using an incubated broth culture of the isolate. A loopful of each culture was placed at the centre of the cover slip such that the culture loop was in the centre of a clean grease free glass slide and pressed firmly so that the oil seal the cover in position. The slide was then carefully and quickly inverted again such that the cover slip was on the top and the culture drop now appeared hanging. It was then observed under low power objective X40 lens. The mobile cells are seen moving rapidly in the field. Besides, a culture of *paenibacillus* will swim on a non-selective agar.

Biochemical Test

Oxygen Utilization Test, sugar fermentation and sugar test were carried out to further characterize *Paenibacillus spp.*

Oxygen Utilization Test

Some organisms are aerobic, anaerobic, facultative anaerobes or microaerophilic, the oxygen utilization test shows the oxygen relationship of different organisms. Mueller Hinton agar was prepared and dispersed into mcCarthney bottles and autoclaved after which the medium was placed on the work bench and allowed to solidify at room temperature. An inoculating loop was used to make a stab culture on the media of the isolate by deeping into the bottom of the bottle at this point the bottles were incubated at 37 °C for 24-48hrs in an Anaerobic Jar (Biomerieux, France) in N_2(80%), H_2(10%) and CO_2 (10%).

It was observed that all the isolates grew both at the top and at the bottom of the culture which is a characteristic of facultative anaerobes.

Sugar Fermentation Test

Most microorganisms are capable of metabolizing a wide variety of sugars as energy sources. This metabolizing ability was demonstrated by inoculating a colony of microorganism into test tubes containing triple sugar ion agar (TSI), which was prepared by dissolving 15grams of TSI into 100ml of distilled water. 9ml of the TSI was dispensed into the test tubes. After solidifying, the isolate was stabbed into the agar and inoculated at 37^{o}C for 72 hours. It was observed for sucrose, glucose, lactose, and gas and hydrogen sulphide production. When lactose, glucose and sucrose were present, there was a change in colouration to yellow. If there was a space at the bottom or middle of the test tube, gas was present while dark colouration indicates that hydrogen sulphide was produced.

Also 1gram of maltose and arabinose was dissolved in 250ml of sterile distilled water containing nutrient broth with phenol red as indicator and autoclaved. It was dispensed in aliquots into McCartney bottles and an inverted Durham tube and the test organism inoculated. A change in colour from red to blue showed the fermentation of the organism and a space at the bottom of the inverted Durham tube showed that gas was produced during the fermentation of the sugar.

Confirmatory Tests

Confirmatory tests was carried out to further confirm the presence of *paenibacillus,* aerotolerance test was done for all the positive isolates.

Aerotolerance Test

Facultative anaerobe also thrive and has same biochemical metabolism like obligate anaerobes but it can grow in the presence of oxygen, this is the reason why this test was carried out as a confirmatory test to distinguish between facultative anaerobes and strict anaerobes.. A culture of the isolate was inoculated aerobically and incubated at 37^0C for 24 hours and the growth pattern was studied. Organisms that did not grow under aerobic conditions were discarded because *Paenibacillus* is a facultative anaerobe.

Molecular Analysis

DNA extraction

DNA Extraction was carried out on the samples using the Jena Bioscience Bacteria DNA Preparation Kit (Germany), according to manufacturer's instruction.

PCR Amplification of the 16SrRNA gene (27F and 1492R)

Polymerase chain reaction was carried out to amplify the 16SrRNA gene of the bacteria using the primer pair 27F-5'-AGAGTTTGATCCTGGCTCAG-3', and 1492R 5'-GGTTACCTTGTTACGACTT -3'. The PCR reaction was carried out using the Solis Biodyne 5X HOT FIREPol Blend Master mix. PCR was performed in 25 µl of a reaction mixture, and the reaction concentration was brought down from 5x concentration to 1X concentration containing 1X Blend Master mix buffer Buffer (Solis Biodyne), 1.5 mM $MgCl_2$, 200µM of each deoxynucleoside triphosphates (dNTP) (Solis Biodyne), 25pMol of each primer (Jena Bioscience, Germany), 2 unit of Hot FIREPol DNA polymerase (Solis Biodyne), Proofreading Enzyme, 5µl of the extracted DNA, and sterile distilled water was used to make up the reaction mixture. Thermal cycling was conducted in a Peltier thermal cycler (PTC100) (MJ Research Series) for an initial denaturation of 95°C for 15 minutes followed by 35 amplification cycles of 30 seconds at 95°C; 1 minute at 61°C and 1 minute 30 Seconds at 72°C. This was followed by a final extension step of 10 minutes at 72°C.

Agarose Gel electrophoresis

Electrophoresis of the DNA was carried out on a 1.5% agarose gel in a 0.5X concentration of Tris-Borate-EDTA (TBE) buffer. Agarose gel was prepared by boiling 1.5g of agarose powder in 100ml of 0.5X TBE buffer. After boiling, the solution was allowed to cool and 10µl of ethidium bromide was added to the cooled agarose solution. This was poured into a casting tray with a comb placed 2 meter from the rim to form wells. The gel was allowed to set for 30 minutes and the comb was gradually removed. 5µl of the amplified PCR products were then loaded into the wells. A DNA 100bp molecular weight marker (Solys biodyne) and a negative control (ultra pure water) were also loaded into one of the wells. The gel was thereafter electrophoresed in a horizontal tank at a constant voltage of 80V for about 1 hour 30 minutes. After electrophoresis, DNA bands were visualized by ethidium bromide stainingnunder a short wave ultraviolet light transilluminator and the photograph were taken using a digital camera.

Sequencing

All PCR products were purified and sent to Inqaba (South Africa) for Sanger sequencing. The corresponding sequences were identified using the online blast search at, http://blast.ncbi.nlm.nih.gov/Blast.cgi

Plasmid analysis

Plasmid extraction was done using the mini prep TENS method as described by Zhou *et al*., 2016 with slight modification. Overnight bacterial culture of 1.5 ml was obtained and then spun for 10s in a micro-centrifuge (10,000 rpm) to pellet cells. The supernatant was gently decanted, leaving 50 - 150 µl together with cell pellet, this was vortexed for 10 seconds at high speed to re-suspend cells completely. Then 300 µl of TENS was added. Then, the solution was mixed by inverting tubes for 5 - 6 times until the mixture became sticky and white precipitates forms. The samples were then incubated in ice for 10 minutes to prevent the degradation of chromosomal DNA. Thereafter, 150 µl of 3.0 M sodium acetate (pH 5.2) was added to stop lysing process, then,

it was vortexed for 1 min to mix completely. The mixture was spun for 10,000 rpm for 5 min to pellet cell debris and chromosomal DNA .Then, the supernatant was transferred to a fresh eppendorf tube, where it was mixed with 900 µl of 100 % ice-cold. Then, spun for 2minutes to precipitate the plasmid DNA and RNA (white pellet is observed) from the supernatant. The supernatant was discarded, the pellet was rinsed twice with 1 ml of 70% ethanol and the pellet was air dried. Pellet was then re-suspended in 20 – 40 µl of TE buffer. The plasmid DNA was separated on a 0.8% agarose gel and electrophoresis was carried out at 80V for 1 hour 30 minutes as described above. After electrophoresis, DNA bands were visualized by ethidium bromide staining. Lamda DNA Hind III ladder was used as plasmid DNA molecular weight standard.

Storage of Isolates

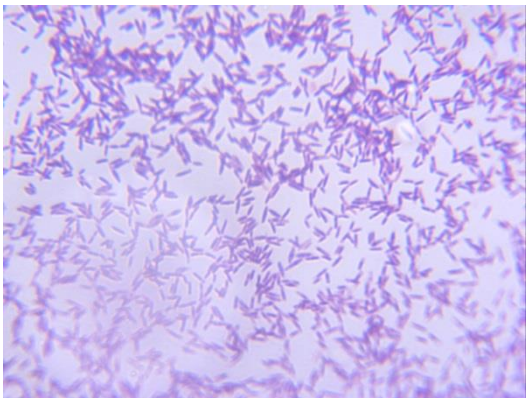
To maintain a stock of isolate strains of *paenibacillus spp* at -20^{0}C, an individual isolated colony was picked from the FAA agar plate using a sterile inoculating loop and suspended in 10 ml of pre-reduced cooked meat medium supplemented with 0.03% L-cysteine. It was then incubated overnight anaerobically at 37^{0}C, or until the culture becomes turbid. A 50% glycerol broth was prepared by mixing 50% glycerol with 50% Mueller Hinton broth and dispensed in aliquots into cryovials. It was autoclaved at 121^{0}C for 15 minutes at 15psi.

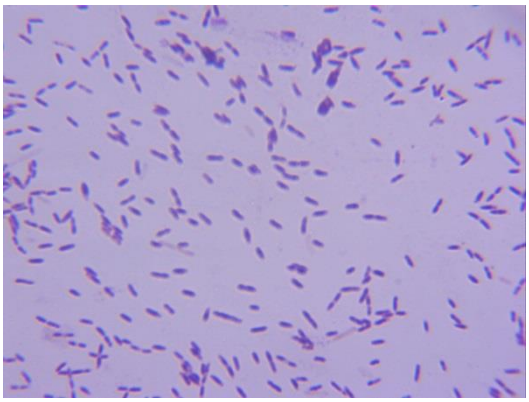
0.5ml of the culture was added to the 1.8 ml cryogenic tube after cooling to create a 40% glycerol stock of the isolated strain. The cryogenic tube was tightly capped, mixed well and placed in an anaerobic jar overnight this is to allow growth of the organism and incorporation of the glycerol broth into the cell wall of the organism afterwards it is removed from the anaerobic jar and placed in a -20^{0}C freezer for long term storage.

RESULTS

A total of 138 faecal samples were analyzed using standard aerobic and anaerobic culture methods. A total of 35(25%) isolates were positive with the following morphological characteristics, shiny, smooth with highly irregular forms and were identified as Gram positive bacilli with endospore (figure 1). The total varied isolation rates between both farms was 23(34.8%) in Oke-Aro farms and 12(16.7%) in Otta farms. The distribution rates among different categories in each farm included; Boars 1(9.1%), Sows 2(13.3%), Growers/Guilts 4(22.3%) and Weaners/suckers 5(17.9%) for Otta farms and Boars 3(37.5%), Sows 5(33.3%), Growers/Guilts 6(22.2%) and Weaners/Suckers 9(39.1%) for Oke-Aro farm (table 1). Oke Aro farm recorded a prevalence of 34.8% while Otta farm had a prevalence rate 16.7%.

Isolates with similar biochemical test activities were grouped into 7 categories; groups A, B, C and G were isolates from Oke-Aro farm while groups D, E and F were isolates from Otta farm table 2. Table 3 shows representatives of DNA sequencing from each farm using 16sRNA gene amplification. The species identified includes; *Paenibacillus konsidensis*, *Paenibacillus massiliensis, paenibacillus timonensis, Paenibacilus terrae* (Oke-Aro farm), *Paenibacillus amyloticus, Paenibacillus polymyxa, Fontibacillus phaseoli* (Otta farm) with a range of 95-99% identity.The evolutionary history (phylogenic tree) was inferred using the Neighbour-Joining method to determine the relationship between strains as shown in figure 4.





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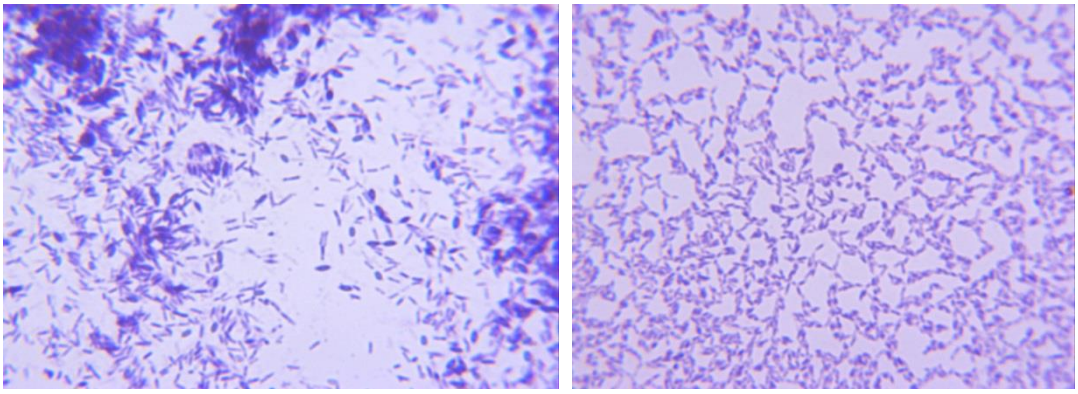

Figure 1: Gram stain microscopy of *Paenibacillus* species showing Gram positive bacilli with endospores.

Table 1: Distribution of *Paenibacillus* species isolated from both farms

Pigs	Otta farm			Oke Aro farm		
Category of pigs	No. of samples collected	No. of *Paenibacillus spp* isolated	Prevalence (%)	No. of samples collected	No. of *Paenibacillus* spp isolated	Prevalence (%)
Boars	11	1	9.1	8	3	37.5
Sows	15	2	13.3	15	5	33.3
Growers/Guilts	18	4	22.2	20	6	30.0
Weaners/Suckers	28	5	17.9	23	9	39.1
Total	72	12	16.7	66	23	34.8

KEY: Boars – adult male (30 weeks & above)
Sows – adult female (10 weeks & above)
Growers – Female (8 – 16weeks)
Guilts – Female (16 – 42 weeks)
Weaners – piglets (12 weeks)
Suckers – piglets (1-8 weeks

Table 2: Groups of isolates with similar biochemical activities

	NUMBER OF ISOLATES IN SAME GROUP WITH SIMILAR TEST ACTIVITIES						
BIOCHEMICAL TEST	**7 Group A**	**9 Group B**	**4 Group C**	**1 Group D**	**5 Group E**	**8 Group F**	**2 Group G**
motility	+	+	+	+	+	+	+
anaerobiosis	+	+	+	+	+	+	+
Starch hydrolysis	+	-	+	+	+	+	+
catalase	-	-	+		+	+	+
Oxidase activity	-	-	-	+	-	-	-
Hydrogen sulphide production	-	ND	ND	ND	-	+	ND
Vogues proskeur	-	-	-		-	+	ND

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Indole	-	ND	ND	ND	-	-	ND
Spore formation	+	+	+	+	+	+	+
Nitrate reduction	+	-	+	+	+	-	+
Acid production test: Glycerol	-	-	+	+	+	+	+
Ribose	+	-	+	-	+	+	+
Xylose	-	+	+	-	-	+	+
Inulin	-	+	-	-	+	+	+
Sucrose	+	+	+	-	+	+	+
Mannose	+	-	+	-	+	+	+
Mannitol	-	+	+	-	+	+	+
Glycogen	+	-	+	+	+		+
Gluconate	+	-	-	+	-	ND	+
Arabinose	-	-	-	-	+	-	-

KEY: + positive reaction, - negative reaction, and ND not determined

Table 3: List of bacteria isolates identified by 16sRNA gene sequence analysis.

Genus	GROUP OF ISOLATES	Collection sites	Genbank accession number	Blast Value	% ID
Paenibacillus konsidensis	A	OKE-ARO FARM	NR 044395.1	0.0	99%
Paenibacillus massiliensis	B	OKE-ARO FARM	NR 115175.1	0.0	97%
Paenibacillus timonensis	C	OKE-ARO FARM	NR 115198.1	0.0	95%
Fontibacillus phaseoli	D	OTTA FARM	NR 118663.1	0.0	99%
Paenibacillus polymyxa	E	OTTA FARM	NR 117723.2	0.0	98%
Paenibacillus amyloticus	F	OTTA FARM	NR 025882.1	0.0	95%
Paenibacillus terrae	G	OKE-ARO FARM	NR 025170.1	0.0	99%

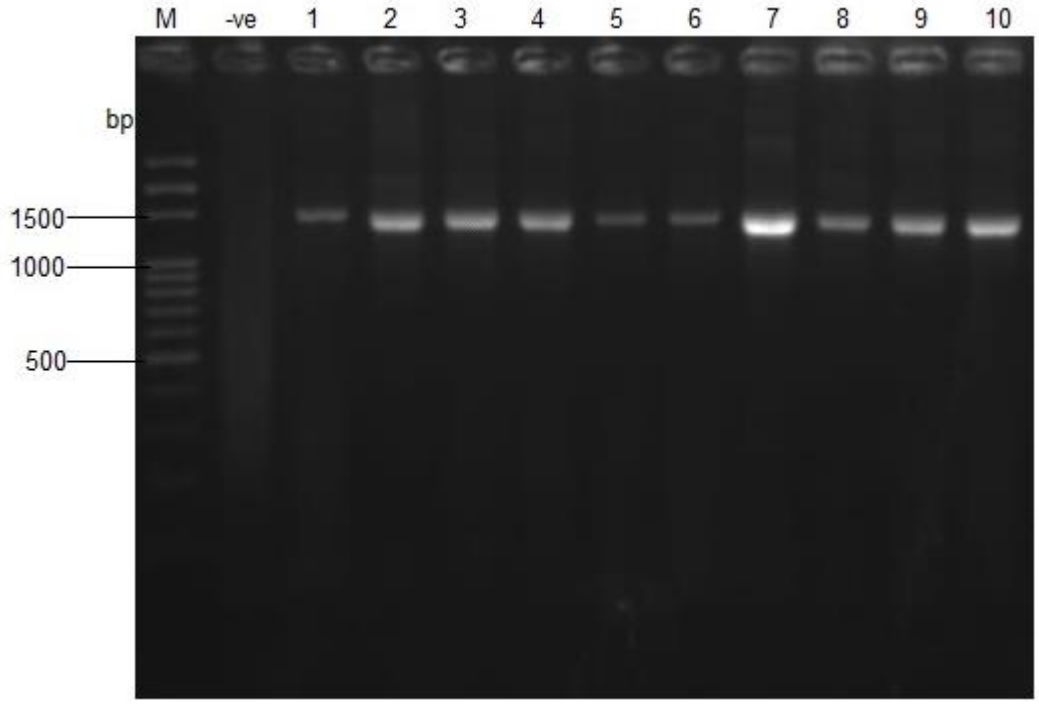

Figure 3: Agarose gel electrophoresis (1.5%) showing lane 1-10 PCR amplified products of 1500bp. –ve is negative control of PCR mix without DNA and M is a 100 base pair ladder.

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PHYLOGENIC TREE OF *PAENIBACILLUS SPECIES*

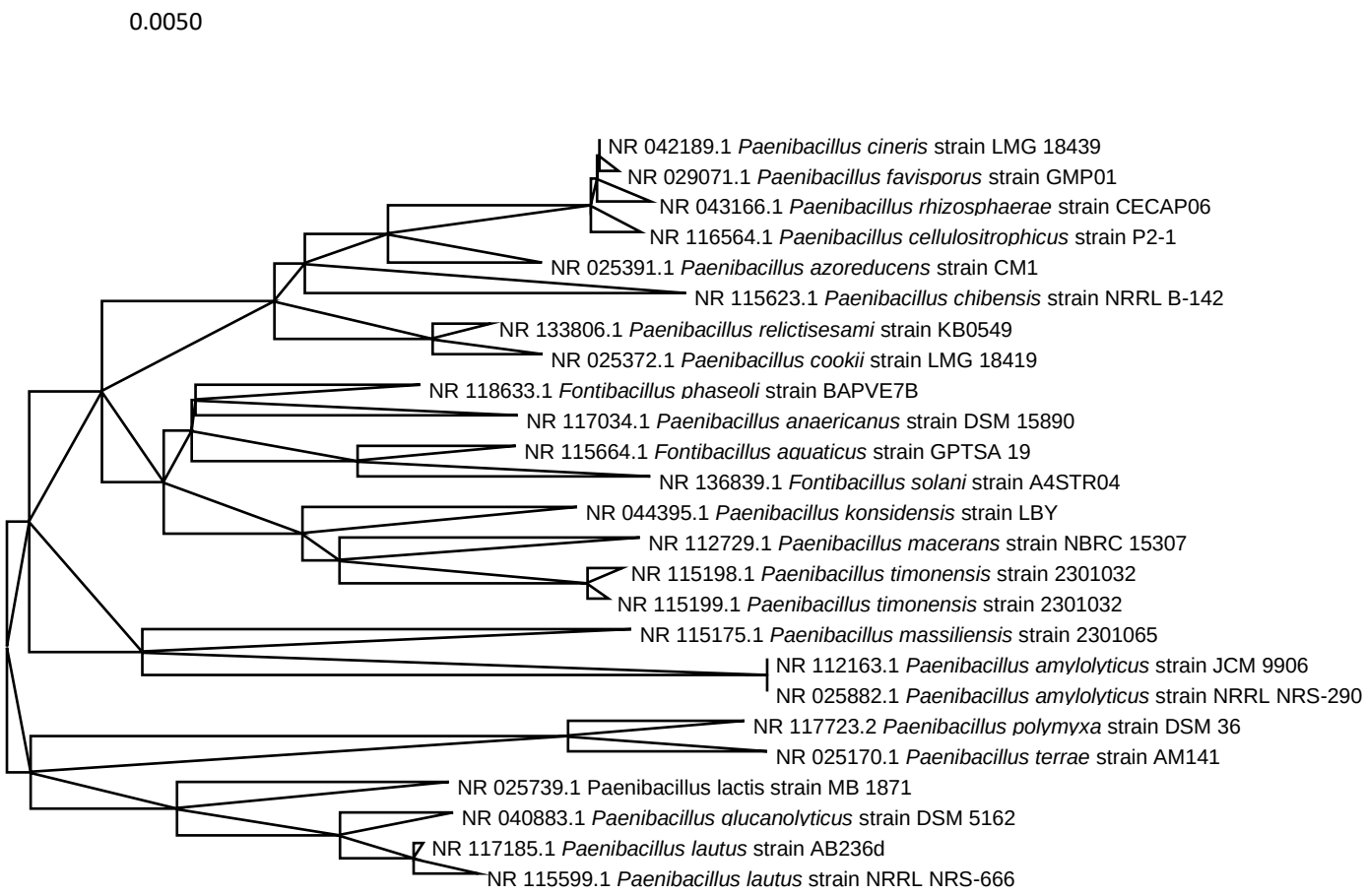

Figure 4. Evolutionary relationships of taxa

The evolutionary history was inferred using the Neighbor-Joining method based on 16S rRNA sequences. The optimal tree with the sum of branch length = 0.27833917 is shown. All positions containing gaps and missing data were eliminated. There were a total of 1328 positions in the final dataset. Evolutionary analyses were conducted in MEGA7.

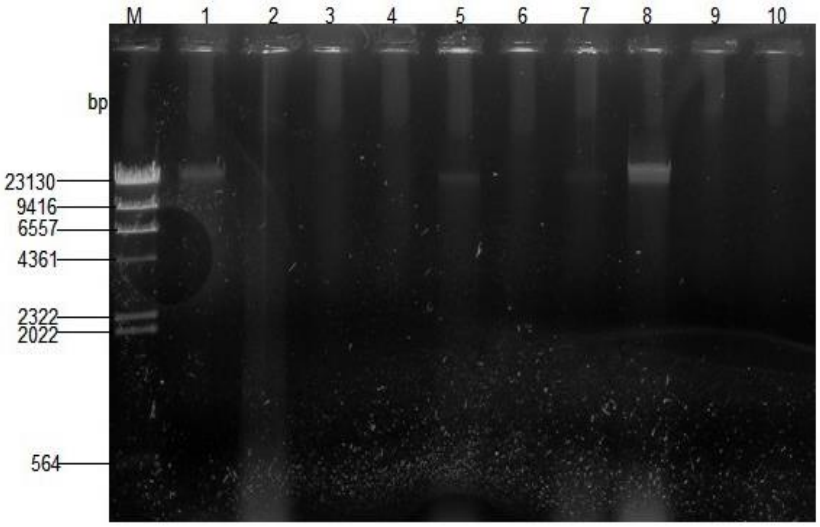

Figure 5: Agarose gel electrophoresis showing the presence of plasmid in lane 1, 5, 7 and 8 with Lane M as Lamda Hind III molecular weight marker.

DISCUSSION

In this study, a total of 35(25%) out of 138 samples collected were positive for *paenibacillus species*, Unfortunately, this positive rate only represents a fraction of the total epidemiology due to lack of veterinary suspicion in pig farms in Nigeria. The distribution of isolates included 25% (*n*=35) of total pig samples being positive for the presence of *paenibacillus species*. Oke-Aro farm recorded a prevalence of 34.8% while 16.7% prevalence was recorded in Otta farm, This was however due to differences in sanitation conditions, environmental conditions and veterinary practices between the two farms as it was observed that the cleaning procedure in Otta farm was properly managed than Oke-Aro farm, in line with a report by Hecht (2006) which showed that higher sanitary conditions limits contamination and spread of anaerobic spore formers like *C. difficile.*

The isolation of *paenibacillus* from pig feces in this study is also in line with a report by Khan and Edward (2001), who reported the presence of this organism in swine fecal slurry storage.

The identity of this microbe was determined by biochemical test and was further confirmed by molecular analysis using 16sRNA sequencing. Although, *Fontibacillus* was also identified *Paenibacillus* was most frequent, species identified included; *Paenibacillus konsidensis*, *Paenibacillus massiliensis, Paenibacillus timonensis, Paenibacillus terrae* from Oke-Aro farm and *Paenibacillus phaseoli, Paenibacillus polymyxa, Paenibacillus amyloticus, Fontibacillus phaseoli* from Otta farm with a range of 95-99% identity.

P*aenibacillus spp* being an opportunistic human pathogens, several of which have been isolated from humans globally (Grady *et al*., 2016) has been associated with diseases such as chronic kidney disease (Padhi *et al*., 2013), premature birth (Deleon and Welliver, 2016), whipple disease (Roux *et al*., 2008), skin cancer, chronic interstitial nephropathy and acute lymphoblastic leukemia (Roux *et al*., 2004). Previous studies have reported the isolation of different strains of this organism in patients undergoing haemodialysis, prosthetic joint infection, bacteremia in cerebral infection, Carcinoma intestinal nephropathy and leukemia (Nasu *et al*., 2003, Roux *et al*., 2004, Ko *et al*., 2008, and Ouyang *et al.,* 2008).

Conclusion and Recommendation

Although *peanibacillus* has been implicated in all these infections, no study has linked these organism in gastrointestinal infections in animals and humans, hence the concern for the result of this study which seem to have implicated *peanibacillus* in diarrhoeic stool from pigs, prior to this study many of the piglets suffered diarrhoeic infection of which over 75% of them died and the cause was not determined due to inadequacy/ difficulty in accessing laboratory materials for the analysis of anaerobic infection in animal farms in Nigeria. Hence further studies is required to determine the role of *peanibacillus* in gastrointestinal infections in pigs.

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