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ASSESSING BACTERIAL DIVERSITY IN TROPICAL BOVINE COW DUNG THROUGH ILLUMINA SEQUENCING

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Abstract

Cow dung is the undigested remnant of plant matter of *Bos indicus* (cow), consisting of cellulose and lignin, hemicellulose, crude protein, and minerals. Despite its numerous benefits and due to the use of cultural base techniques, little is known about cow dung microbiota because of the shortcomings associated with the use of conventional culture methods. This has necessitated the use of a robust high-throughput technique to determine the bacterial community of cow dung. Total DNA was extracted from a fresh cow dung sample, and the bacterial 16S rRNA community was sequenced via the Illumina platform. The bacterial phyla identified included *Firmicutes* (51.12%), *Proteobacteria* (36.53%), *Actinobacteria* (11.44%) and another unclassified group (0.92%). The most predominant class was *Bacilli* (49.71%) followed by *Proteobacteria* (gamma-type 29.85%), *Actinobacteria* (11.44%) and the least abundance was recorded by *Proteobacteria* (alpha-type 6.47%). The common genera in this group included *Staphylococcus* (46.31%), *Legionella* (13.48%), *Micrococcus* (9.42%), *Acinetobacter* (5.53%), *Alishewanella* (5.00%), *Pseudomonas* (5.22%), *Paracoccus* (4.21%) with 10.33% of the group yet to be classified. This study uncovers a high diverse bacteria community in the examined cow dung that could be harnessed for usage in different areas such as medicine, agriculture, and industry.

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Introduction

Cow dung is the undigested solid waste of *Bos indicus* (cow). It consists of cellulose, lignin, hemicellulose, crude protein, and minerals such as nitrogen, potassium, sulfur, cobalt, iron, magnesium, calcium, etc. It is a mixture that consists of feces and urine, usually in a ratio of 3:1 (RANDHAWA and KULLAR 2011). Cow dung as a community is rich in diverse types of microorganisms, such as bacteria, fungi, yeast, and protozoa. Some different genera of bacteria that have been reported in cow dung include *Klebsiella oxytoca, Klebsiella pneumoniae*, *Enterobacter aerogenes, Escherichia coli, Morgarella morganii, Pasteurella spp., Providencia alcaligenes,* and *Pseudomonas* spp. (SAWANT et al. 2007). Cow dung is more than just a waste because of its diverse applications including medicine, environmental management, energy sources, and agriculture. Cow dung possesses antiseptic and prophylactic or disease preventive properties (THENMOZHI et al. 2018). It destroys the microorganism that causes disease and putrefaction. Medicinal properties of five products collectively known as panchgavya obtained from cow namely milk, ghee, curd, dung, and urine are supported by their use in the preparation of various herbal medicines (PATHAK and KUMAR 2003, JARALD et al. 2008). Possible applications of cow dung microorganisms in pharmaceutical industry have been indicated by TEO and TEOH (2011) and it was shown that isolate K4 possessed antibacterial activity against *E. coli*. Research has also been conducted on water, ethanol, and n-Hexane extract of whole cow dung against *Candida*, *E. coli*, *Pseudomonas* and *Staphylococcus aureus* by SHRIVASTAVA et al. (2014) revealing their antimicrobial properties. Cow dung is also used as a co-product in agriculture, such as manure, biofertiliser, biopesticides, pestrepellent and as a source of energy (DHAMA et al. 2005). LI et al. (2009) reported 67 ml/g methane yield from anaerobic digestion of cow manure, whose total and volatile solids were 23.4 and 13.8 g/l, respectively. Thus, cow dung may not only act as a substitute for chemical fertilisers because it supplements organic matter, but also as a conditioner for soil (GARG and KAUSHIK 2005, YADAV et al. 2013, BÉLANGER et al. 2014). As reported by VAKILI et al. (2015), adding cow dung to palm oil biomass improves the compost's physical and chemical properties, as well as its nutritional composition. ARSLAN et al. (2008) and VAKILI et al. (2015) recognize that compost improves soil nutrients and water retention. Among the microorganisms present in cow dung are *Acinetobacter*, *Bacillus*, *Pseudomonas*, *Serratia*, and *Alcaligenes* spp., making them suitable for biodegradation of pollutants (ADEBUSOYE et al. 2007, UMANU et al. 2013). Thus, cow dung may not only act as a substitute for chemical fer-

tilisers because it supplements organic matter, but also as a conditioner for soil (GARG and KAUSHIK 2005, YADAV et al. 2013). Due to its richness in microbial diversity, cow dung had been employed in removing toxic pollutants from the environment (UMANU et al. 2013). Cow dung slurry maintained in the ratio of 1:10 or 1:25 is able to degrade the rural, urban and hospital wastes, including oil spillage to five basic elements (RANDHAWA and KULLAR 2011). A study by ORJI et al. (2012) highlights the importance of cow dung isolates, both bacterial and fungal, for reducing total petroleum hydrocarbons to 0 % in polluted mangrove soil. UMANU et al. (2013) suggested that the application of cow dung in an appropriate concentration may prove very efficient in biodegradation of water contaminated with motor oil. WYSOCKA-CZUBASZEK (2019) investigated by comparing the influence of digestate addition on soil chemical properties with traditional organic fertilizers such as liquid and solid cattle manure and with mineral fertilizer. Finding by the same author was that the digestate supplied soil with a significant amount of NH_4-N, whose nitrification was slower comparing to soils treated with mineral fertilizer and liquid cattle manure.

Because microorganisms can be easily cultivated, microbial enzyme application in industry is on the rise. The microbial diversity of cow dung makes it a suitable source of important industrial enzymes. One member of the xylanolytic bacteria *Paenibacillus favisporus* from cow dung has been reported to produce various types of hydrolytic enzymes such as xylanases, cellulases, amylases, gelatinase, urease, and β-galactosidase (ENCARNA et al. 2004). In addition, cow dung can also serve as a suitable substrate to produce enzymes (VIJAYARAGHAVAN et al. 2012). It is also used in the production of biogas as an alternative source of renewable energy. Biogas, a mixture of different gases produced by anaerobic fermentation of organic matter from methanogenic bacteria, mainly constitutes methane (50–65%) and CO_2 (25–45%) (SHARMA 2011).

The mycelial growth of *Sclerotinia sclerotiorum* has been reported by BASAK et al. (2002) to be suppressed by cow urine and cow dung. The fungus is one of the most common pathogens that cause sclerotina rot in cucumber and other vegetables. Similarly, BASAK and LEE (2001) also reported that fresh cow urine and cow dung have inhibitory action on mycelial growth of *Fusarium solani*, f. sp. *cucurbitae* and *F. oxysporum* f. sp. *cucumerinum*, which are responsible for *Fusarium* root rot and wilt of cucumber. *Bacillus subtilis* isolated from cow dung has been reported to have antagonistic activity against plant diseases (GUPTA et al. 2016). The bacterium can be employed as a biocontrol agent and due to the high heat tolerance of *Bacillus* sp., it can be employed industrially to produce amy-

lase (RAMACHANDRAN et al. 2016). *Mycobacterium vaccae*, a nonpathogenic bacterium first isolated from cow dung, possesses antidepressant properties. When inhaled, it enhances the growth of neurons, which stimulates the production of serotonin and norepinephrine in the brain (LOWRY et al. 2007). Smoke generated from burnt cow dung is also reported to be effective as a mosquito repellant (GUPTA et al. 2016).

Cow dung has also been employed over the years as organic manure in agriculture which serves as an alternative to fertilizer. According to the US Department of Agriculture, in California alone, up to 60 million tons (at 30 kg/head) of animal waste are produced per year by 5.2 million cattle and calves, and a large portion of this is waste used as manure in cropland (USDA 2016). This has reduced the use of chemical fertilizers that are not eco-friendly. Some of the microbiomes found in dung can promote plant growth. Zinc, a micronutrient required for plant growth and productivity has been reported to be solubilized by *Bacillus* sp. isolated from cow dung. This implies that cow dung is rich in microorganisms that can be used as a bioinoculant. Some of these cow dung bacteria have also been implicated in phosphorus solubilization, and siderophore, HCN and indole acetic acid (plant hormone) production (KALPANA and DINESH 2019).

The utilization of cow dung as a fertilizer is associated with negative consequences. This is mostly due to the prevailing practice of disposing of cow dung in piles, slurries, or lagoons, which leads to significant greenhouse gas emissions of methane, environmental degradation, negative health impacts, and loss of valuable nutrients that could otherwise be utilized to enhance soil fertility. It is essential to consider alternative methods of disposal and explore environmental friendly options for the utilization of cow dung as a fertilizer. This will not only mitigate the negative impacts but also provide a valuable source of nutrients to improve soil health and fertility. The high level of pathogens in surface water has been attributed to run-off from farmland into surface water (PANDEY et al. 2014). In addition, harmful pathogens in untreated manure can contaminate crops and vegetables on farmland (ERICKSON et al. 2014, RAYMOND et al. 2021).

Despite this wide application of cow dung, little is known about the cow dung microbiota. This lack of knowledge may be due to the method used to analyze the cow dung community. Cultural-dependent methods have been used to study cattle intestinal microflora. In addition, this method is time consuming, and only approximately 1% of bacteria have been successfully cultured, necessitating metagenomic analytical techniques. The remaining 99% is yet to be cultured; hence the need for metagenomics approaches (RADA et al. 2006). This study aimed to analyze the bacterial diversity of cow dung from a tropical *Bos indicus* using Illumina

sequencing technology. This study delves into the depths of bacterial diversity found within tropical *Bos indicus* cow dung. The results promise to offer an enlightening glimpse into the microbial world thriving within this unique ecosystem.

Materials and Methods

Sample collection and DNA extraction

Fresh cow dung was collected from a cattle farm in Gbagada, Lagos Nigeria and transported immediately in an ice pack (4ºC) to Africa Bioscience Laboratory, Ibadan, Oyo State, Nigeria for community DNA extraction. The cow dung properties had been previously determined by ADEGUNLOYE and ABE (2020). DNA extraction from the cow dung sample was carried out using a Presto Soil DNA Extraction kit (Geneaid Biotechnology Limited, Taiwan) according to the manufacturer's instructions and sent to Xcelris Genomics Laboratory Gujarat, India for Illumina sequence analysis. The quality of gDNA was checked on a 0.8% agarose gel (loading 5 µl of the sample) for the presence of intact bands at 110 V for 30 mins. The sample (1 µl) was loaded in a Thermo NanoDrop ND-1000 UV/VIS Spectrophotometer (Thermo Fisher Scientific, UK) to determine the A260/280 ratio. The DNA was also quantified using a Qubit dsDNA HS Assay kit (Life Technologies, Madison, USA). One microliter of each sample was used for determining concentration using Qubit® 2.0 Fluorometer.

Library preparation

The amplicon library was prepared using the Nextera XT Index Kit (Illumina Inc., San Diego, CA, USA) according to the 16S metagenomic sequencing library preparation protocol (Part #15044223 Rev. B). Primers for the amplification of the V3-V4 hypervariable region (V3-R: CCTACGGGNBGCASCAG; V4-R: GACTACNVGGGTATCTAATCC) of the 16S rDNA gene of bacteria and archaea were designed by Xcelris Labs Limited, Gujarat, India and synthesized in Xcelris PrimeX facility. The amplicons, together with the Illumina adaptors, were amplified by using i5 and i7 primers that add multiplexing index sequences, as well as common adapters required for cluster generation (P5 and P7) according to the standard Illumina protocol. The amplicon libraries were purified by 1X AMpureXP beads, checked on an Agilent DNA1000 chip on a Bioanalyzer 2100 and quantified by a Qubit Fluorometer 2.0 using Qubit dsDNA HS Assay kit (Life Technologies, Madison, USA).

Cluster Generation and MiSeq Illumina Sequencing

After obtaining the Qubit concentration for the library and the mean peak size from the Bioanalyzer profile, the library was loaded onto the Illumina MiSeq platform at an appropriate concentration (10–20 pM) for cluster generation and sequencing. Paired-end sequencing allows the template fragments to be sequenced in both the forward and reverse directions on the Illumina platform. The kit reagents were used to bind the samples to complementary adapter oligos on a paired-end flow cell. The adapters were designed to allow selective cleavage of the forward strands after resynthesis of the reverse strand during sequencing. The copied reverse strand was then used to sequence from the opposite end of the fragment.

Sequence data analysis

The generated sequence data were analyzed by QIIME (Quantitative Insight into Microbial Ecology) pipeline with the following workflow: filtering chimeras → OTU (Operational Taxonomic Unit) picking → Taxonomic assignment → Diversity calculation, using the USEARCH61. The forward and reverse sequences were stitched to form longer sequences using PANDAseq (Masella et al. 2012), followed by data cleaning by removal of chimeras using USEARCH61 (*de novo* and abundance-based options). The cleaned data were further subjected to OTU picking, taxonomic assignment and diversity calculation utilizing the UCLUST algorithm within the QIIME pipeline. OTU picking involves the clustering of highly similar sequences together and then the generation of a consensus sequence to represent the cluster. OTUs were picked from the cleaned reads with a benchmark of 97% sequence similarity. Singletons, that is, clusters containing single sequences, were removed from the list of OTUs before taxonomic assignment. Taxonomy assignment was carried out by mapping the OTUs to the Greengenes database at a 90% similarity level.

Results

Alpha diversity

Alpha diversity or within-sample diversity is calculated using an OTU table that gives ideas about species richness. Table 2 summarizes the $\boldsymbol{\alpha}$-diversity, where the columns correspond to alpha diversity metrics and the rows correspond to samples and their calculated diversity measurements. After sequencing, the number of flash reads was 523 117, out of

which 400 590 were non-chimeric sequences, while the number of OTUs was 26 527 (Table 1). The number of OTUs with zero singletons was 3,189. Shannon and Chao1 are the diversity indices used in this study to show alpha diversity. Chao1 focuses on richness, while Shannon shows the number of species and the effect of evenness. The calculated alpha diversity was Shannon (4.70), and Chao1 (3 214.01), and the observed species was 3 189.00 (Table 2).

Table 1

Summary of the OTUs obtained from cow dung

Flash/stitch reads	Non-chimeric sequences	Number of OTUs	Number of OTUs with zero singletons
523 117	400 590	26 527	3 189

Table 2

Alpha-diversity metrics of the bacteria community within a tropical cow dung

Shannon	Observed species	chao1
4.70	3 189.00	3 214.01

Cow dung microbial diversity

The bacteria order *Bacillales* was the most predominant, followed by *Legionellales*, *Actinomycetales*, *Pseudomonadales*, *Alteromonadales*, *Rhodobacterales*, *Rhizobiales*, *Lactobacillales*, *Clostridiales* and an unidentified group (Figure 1). Within the family, the most abundant were *Staphylococcaceae* (46.31%), *Legionellaceae* (13.48%), *Micrococcaceae* (9.42%), *Moraxellaceae* (5.55%), *Chromatiaceae* (5.50%), *Pseudomonadaceae* (5.22%), *Rhodobacteraceae* (4.21%) and other belonging to unclassified group (15.81%) – Figure 1. The phyla *Firmicutes*, *Proteobacteria* and *Actinobacteria* were fully represented in the cow dung community (Figure 2). Predominantly, *Firmicutes* constituted 51.12% followed by *Actinobacteria* 11.44%, *Proteobacteria* 36.53% and other unclassified group (0.92%) of the entire population (Figure 2). The most predominant class was *Bacilli* (49.71%) followed by *Proteobacteria* (Gamma – 29.85%), *Actinobacteria* (11.44%) and *Proteobacteria* (Alpha-6.47%) had the least abundance (Figure 2).

In this study, the common genera included *Staphylococcus* (46.31%), *Legionella* (13.48%), *Micrococcus* (9.42%), *Acinetobacter* (5.53%), *Alihewanella* (5.00%), *Pseudomonas* (5.22%), *Poracoccus* (4.21%) (Figure 3). *Staphylococcus* (46.31%) was the most predominant followed by *Legionella* (13.48%), *Micrococcus* (9.42%), *Acinetobacter* (5.53%, *Alihewanella* (5.00%),

Pseudomonas (5.22%) and *Poracoccus* (4.21%) had the least abundance. However, there was 10.33% abundance by the group yet to be classified (Figure 3).

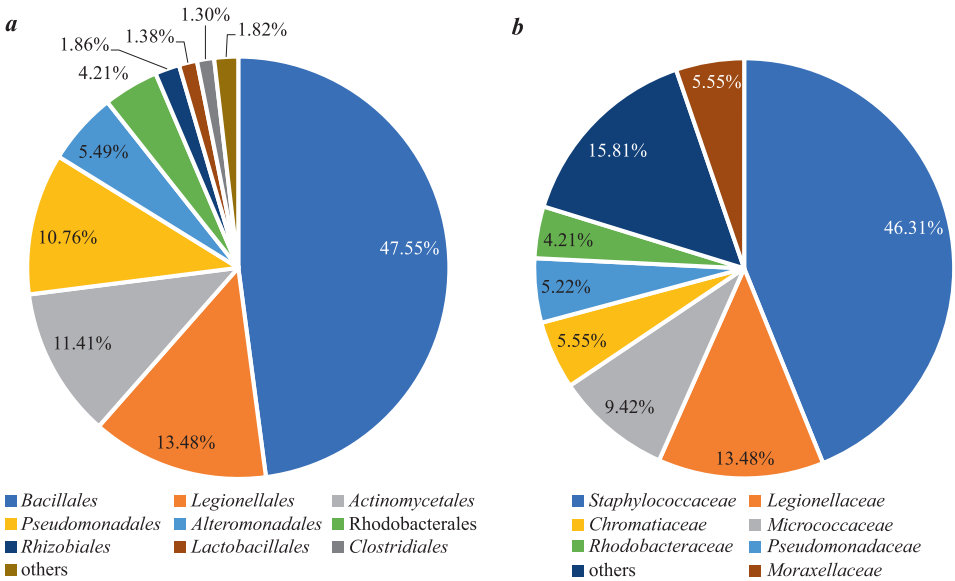

Fig. 1. Taxonomic distribution of bacteria from a tropical cow dung at the order (*a*) and family (*b*) levels

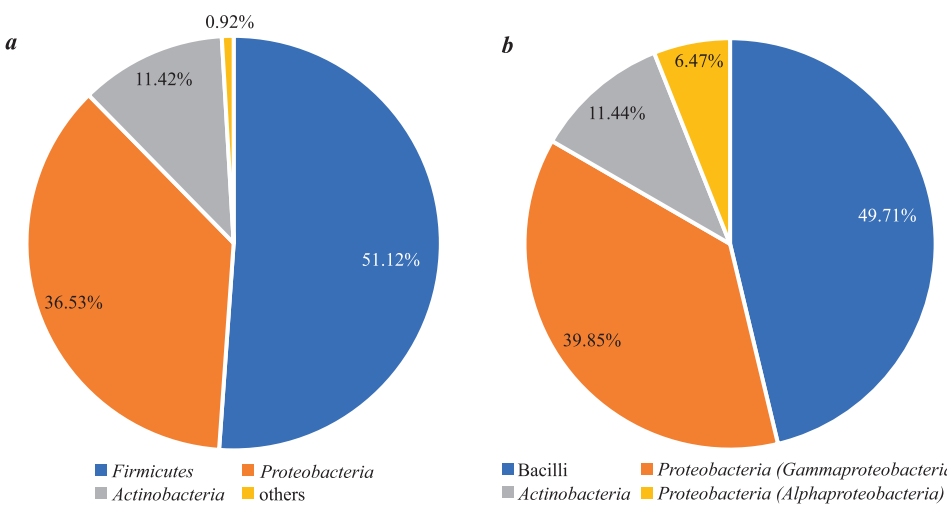

Fig. 2. Taxonomic distribution of bacteria from a tropical cow dung at the phylum (*a*) and class (*b*) levels

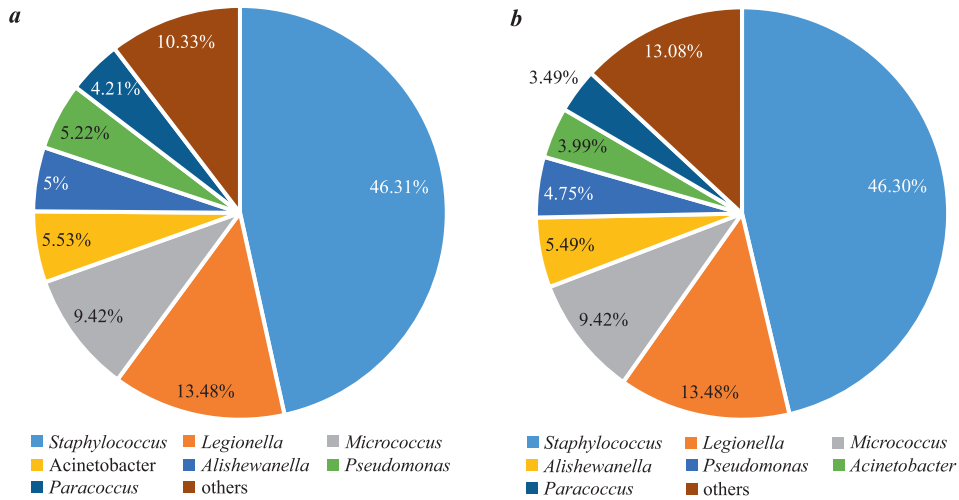

Fig. 3. Taxonomic distribution of bacteria from a tropical cow dung at the genus (*a*) and species (*b*) levels

Discussion

Knowledge of the composition, diversity and structure of the microbial community was limited until the emergence of fingerprinting techniques and subsequent molecular approaches. These new techniques broaden scientists' understanding of the relationship between microbes and their environment (GREEN et al. 2008). The bacterial diversity of cow dung had in the past been assessed by a culture-dependent method. However, according to GIRIJA et al. (2013), culture-based methods show bias to facultative anaerobes because they can be easily cultured under laboratory conditions, while strict anaerobes are underestimated because they fail to grow under normal growth conditions. Previous studies by the same authors reports the bacterial diversity and phylogenetic relationship of indigenous bacteria of cow dung by 16S rRNA gene libraries (GIRIJA et al. 2013). MTSHALI et al. (2022) studied the bacterial communities present in bovine faeces, milk, and blood. Alpha diversity was calculated using the Chao1 index, which measures richness, and the Simpson's and Shannon indexes, which combine richness and evenness. (GOTELLI and COLWELL, 2001, HOQUE et al. 2020). Alpha diversity values were significantly higher in feces than in milk or blood. This present study obtained a Shannon of 4.70, which was lower than the 6.7251 recorded by MTSHALI et al. (2022) for bovine faeces, while this study obtained a Chao1 of 3214.01, which was higher than 1710.5 recorded by MTSHALI et al. (2022).

Bacteria belonging to the phyla *Bacteriodetes* (38.3%), *Firmicutes* (29.8%), *Proteobacteria* (21.3%), and *Verrucomicrobia* (2%) were identified (GIRIJA et al 2013). In this study we explored the microbial structure and composition of cow dung (bovine faeces) through sequencing of the V3-V4 hypervariable region of the 16S rRNA gene, employing the Illumina Miseq platform. This is an ideal platform for small-scale research due to its cost effectiveness, short turnaround time and comparatively high sequencing depth (GUPTA and VERMA 2019). This study examined the number of reads by only cow dung (bovine faeces) and had a high number of reads. Similarly report by MTSHALI et al (2022) accessed the number of reads returned by sample type and showed that they were disproportionate with the highest number recorded among faeces, followed by milk and blood. The high microbial biomass recorded for faecal samples was anticipated due to the difference in the type of samples being analyzed, for instance, blood samples generally contain low microbial biomass while faecal samples contain high microbial biomass (EISENHOFER et al. 2019).

The most prevalent bacterial groups detected in cow dung samples in this study included members of *Firmicutes*, *Proteobacteria*, *Actinobacteria* and unclassified group. MTSHALI et al (2022) found the most prevalent bacterial groups in the faeces and milk included members of the *Firmicutes* and *Bacteroidota* phyla; while bacterial sequences from blood were predominantly members of *Proteobacteria*, a similar observation to findings of YOUNG et al. 2015. It has been proven that the gastrointestinal tract (GIT) of calves is seeded before birth with a diverse array of microbiota, changing drastically post-partum and successively predominated by *Firmicutes*, *Bacteroidota*, *Proteobacteria* and *Actinobacteriota* in decreasing order of abundance post-weaning (O'HARA et al. 2020, CENDRON et al. 2020, KOESTER et al. 2020, YOUNG et al. 2015, MALMUTHUGE et al. 2015, MAO et al. 2013, OIKONOMOU et al. 2013). In contrast to popular reports however, the *Firmicutes*, *Proteobacteria* and *Actinobacteria* were succeeded by *Verrucomicrobiota* in this study, following a similar microbial distribution pattern to donkey gut microbiota reported by LIU et al. (2014).

The observed faecal microbiota represented a mixture of taxa containing known anaerobic gut microbes (*e.g*., *Clostridium sensu stricto_1*, *Romboutsia* and *Bacteroides*) (CENDRON et al. 2020, DOWD et al. 2008). Initial gut colonizers or bacteria found in the intestine but typically present on other mucosae (*e.g*. *Streptococcus* and *Staphylococcus*) (ALIPOUR et al. 2008); and bacterial genera with potential health effects on cattle and human hosts (*e.g*. *Bacillus* and *Clostridium_sensu_stricto_1*) (DOWD et al. 2008).

It has been reported that the suppression and/or over colonization of certain microbes in a particular niche result in disease pathogenicity, thus emphasizing the need to understand the interaction between the host environment and its inhabiting microbes (DENG et al. 2019). Furthermore, since microbes with zoonotic potential were detected, precaution should be taken to prevent human infection in the sampled community. The routes of infection can be through consumption of contaminated meat and milk; via aerosol due to the proximity of the animal enclosures to their homes; occupational exposure through handling of infected animals as well as aborted foetal material (MAITY and AMBATIPUDI 2021); and most importantly through the unsanitary practices associated with the use of cattle products and by-products by this community (DARIA and ISLAM 2021).

The *Bacillales* order includes several families, such as the *Listeriaceae*, *Planococcaceae*, *Staphylococcaceae*, and *Bacillaceae* (BECKER et al. 2021). Within the family Micrococcaceae, the genus *Staphylococcus* comprises Gram-positive, non-spore-forming cocci that frequently colonize the skin and nasal cavities as common human microorganisms. Five possible human pathogens are found in this genus: *S. aureus*, *S. epidermidis*, *S. saprophiticus*, *S. haemolyticus*, and *S. hominis*; nevertheless, the first three isolates are the most frequently occurring. The capacity to coagulate sets *S. aureus* apart from the other two infections, which are thought to be the most dangerous. In addition to dangerous systemic infections, *S. aureus* can cause several superficial pyogenic (pus-forming) infections of the dermis and underlying tissues. It can produce a variety of toxins, such as poisonous substances, cytotoxins (widely distributed toxins), and enterotoxins (food poisoning). Although they are far less common as pathogens, the other coagulase-negative staphylococci, *S. epidermidis* and *S. saprophiticus*, are occasionally linked to wound infections, endocarditis, and infections in prosthetic joints, to mention a few (BECKER et al. 2021).

Gram-negative bacilli are species of *Legionella*. In the family *Legionellaceae* and the genus *Legionella*, there are currently 42 species of legionellae that have been identified, representing 64 serogroups (BENSON and FIELD 1998). *Legionellae* are Gram-negative bacilli that are thin and somewhat pleomorphic, with a diameter ranging from 2 to 20 µm. It is possible for long, filamentous forms to emerge, especially following growing on agar. *Legionella* is characterized ultrastructurally by having the exterior and inner membranes of Gram-negative bacteria. The majority of its species are motile using a single polar flagellum, and it has pili (fimbriae) (BENSON and FIELD 1998, FANG et al. 1989, WINN 1988, WINN and MYEROWITZ 1981).

The present study identified four genera including *Staphylococcus*, *Legionella*, *Alishewanella* and *Paracoccus*. *Legionella* is the only genus in the family *Legionellaceae*. Fifty species of *Legionella* and more than 70 different serogroups now are recognized (MORONTA 2022). *Legionella* is a genus in the phylum, the majority of which have been isolated only from environmental, rather than from clinical sources. In the environment, these organisms may inhabit complex communities composed of multiple bacterial species that grow within biofilms (MORONTA 2022). They have been isolated from waters with temperatures ranging from 5 to 50 °C; however, they can grow to abundance at the warmer end of this spectrum, particularly in water distribution systems with water heaters (MORONTA 2022). According to BENSON and FIELD (1998), the intricacy of environmental interactions is comparable to that of viral and parasite illnesses. Water is the only known source of *Legionella* species, especially surface waters from lakes, rivers, and drinking water. *Legionella* does not grow in sterile tap water, but it does grow in vitro when free-living amoebae are added (BENSON and FIELD 1998).

Alishewanella is a genus in the phylum *Pseudomonadota* (bacteria). *Gammaproteobacteria* is a class of bacteria in the phylum *Pseudomonadota*. It contains about 250 genera, which makes it the most genus-rich taxon of the prokaryotes (KIM et al. 2009). Several medically, ecologically, and scientifically important groups of bacteria belong to this class *Alishewanella*. There has been paucity of information to the diversity of *Alishewanella* specie. It just recently studies have been explored on this genus (VOGEL et al. 2000). According to NCBI taxonomy, there are 36 isolates from different environments, 27 uncultured *Alishewanella* strains with 16S rRNA gene sequences, and only 5 species of *Alishewanella* with officially published names (VOGEL et al. 2000, KIM et al. 2009, ROH et al. 2009, JUNG et al. 2013). Fermented foods, tidal flat sediments, plant leaf and root surfaces, soils, cold temperature deserts, sludge, permafrost soils, freshwater biofilms, metal tailings, beetle larvae guts, lakes, wastewater, and heavy metal-resistant communities are some isolation sources of the group. These many isolation sources suggest that the *Alishewanella* species are highly adaptable and occupy a wide variety of habitats. Furthermore, a culture-dependent analysis of a lake's bacterial population revealed a prevalence of *Alishewanella* species (POLZ et al. 2013).

The *Paracoccus* genus classification, belonging to the alpha subgroup of *Proteobacteria*, has undergone significant and extensive changes. Numerous new species have been discovered, and the status of existing species has been reevaluated. Currently, the genus comprises 17 species found in diverse environments. Some of these species, including *Paracoc-*

cus alcaliphilus (URAKAMI et al. 1989), *P. carotinifaciens* (TSUBOKURA et al. 1999), *P. aminophilus*, *P. aminovorans* (URAKAMI et al. 1990), and *P. kondratievae* (DORONINA et al. 2002), were isolated from soil. Other species were found in environments containing toxic components, such as *P. alkenifer*, which was isolated from biofilters used in treating waste gases from an animal rendering plant (LIPSKI et al. 1998), *P. methylutens*, which was discovered in groundwater contaminated with dichloromethane (DORONINA et al. 1998), *P. pantotrophus*, which was isolated from sulfide-oxidizing, denitrifying fluidized-bed reactors in plants (ROBERSTON and KUENEN 1983), and *P. kocurii*, which was found in wastewater from semiconductor manufacturing processes (OHARA et al. 1990). Some strains of *P. denitrificans*, the first *Paracoccus* species isolated (BEIJERINCK and MINKMAN 1910), were also found in several different habitats, including sewage, sludge, horse manure, cow dung (LIPSKI et al. 1998), and soil. Bacteria belonging to the *Paracoccus* genus are likely to be essential components of many wastewater treatment system communities (NEEF et al. 1996). Recently, the number of known habitats for these bacteria has expanded, with two new species discovered from the marine environment; *P. seriniphilus* was isolated from the marine bryozoan *Bugula plumosa* (PUKALL et al. 2003, KOESTER et al. 2020), and *P. zeaxanthinifaciens* was isolated from seaweed from the coast of the African Red Sea (ÅRHEM 1989). Moreover, the first *Paracoccus* species associated with human infection (*P. yeei*) was isolated from the dialysate of a patient with peritonitis (TSCHOPP et al. 2013). These findings suggest that these bacteria are more widespread than previously thought.

Conclusion

The characterization of cow faecal microbiota can offer valuable insights into the microbial structure and composition of cow dung samples, particularly in the Nigerian context. By utilizing high throughput sequencing of the V3-V4 hypervariable region of the 16S rRNA gene, the microbiota of faeces from cows was analyzed. The results of the study reveal a novel understanding of the microbial diversity of cow dung samples and can potentially contribute to knowledge acquisition concerning the hypothesized pathway in ruminants. The concurrent detection of microbes in the cow dung samples can offer further insights into the intricate relationships among microbiota, and thus, facilitate a more comprehensive understanding of the microbial ecology of the cow faecal microbiota.

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