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Bull Ibiso
Department of Medical Laboratory Science, Rivers State University, Nigeria

Agi Nkemkanma Vivian
Department of Medical Laboratory Science, Rivers State University, Nigeria

Rhoda ME Nwalozie
Department of Medical Laboratory Science, Rivers State University, Nigeria

Ollor Amba Ollor
Department of Medical Laboratory Science, Rivers State University, Nigeria

Correspondence Author;
Agi Nkemkanma Vivian
Department of Medical Laboratory Science, Rivers State University, Nigeria

The effect of ketogenic diet on gut microbiota of Albino Rat

Bull Ibiso, Agi Nkemkanma Vivian, Rhoda ME Nwalozie and Ollor Amba Ollor

Abstract
The human gastrointestinal tract has the largest number of microorganisms including bacteria, archaea and eukaryotes as compared to other areas of the body. The collection of these microorganisms colonising the gastrointestinal tract is termed the gut microbiota. The human microbial community is strictly influenced by diet, and a good gut microbial community is connected with a better health. The ketogenic diet is a form of diet focusing on reduced intake of carbohydrates and increased intake of fat. Itt has gained popularity as a method to reduce weight and this pattern, by reducing certain type of food, may affect the gut microbiota composition and its related influence on host physiology This study evaluated the effect of ketogenic diet on the gut microbiota of albino rat model. A total of 25 rats were used and divided into 5 groups having 5 rats each. After acclimatization for 2 weeks, group (2, 3, 4 and 5) were fed with ketogenic diet prepared by mixing 65% of standard feed with 35% of commercial butter for 7 and 14 days respectively and their weights recorded. Group 1 rats served as the control group and were fed with pellet and water only throughout the experiment. Stool samples were collected aseptically by pulling from each group into sterile stool sample bottles and sent to the laboratory for immediate culture immediately after acclamitzation, at the end of 7 days and at the end of 14 days respectively. They were analysed using standard convectional microbiological method, moecular ananlysis, total heterotrophic plate count and antibiotic susceptibility were also done. The statistical tool used for analysis was analysis of variance and chi square with $p< 0.05$. The results after measuring the body weight of the rats after acclimatization, 7 days and 14 days for all the groups showed that the body weights of group 1 rats were relatively stable and there was no significant difference. There was weight loss in the groups that were fed with ketogenic diet, with significant difference in group 2 and 3 but no significant difference in group 4 and 5 following a cut off of $p< 0.05$. The heterotrophic plate count of bacteria revealed a decrease in the number of colonies in the groups that were fed with ketogenic diet while the count in group 1 (control) were relatively stable. The bacterial count in all the groups after acclimatization was higher than the count in the groups (2, 3, 4 and 5) that were fed with high fat. Hence, there is decrease in count. It was also observed that the rats that were fed with ketogenic diet had reduced stool mass. The rate of resistance to the antibiotics used for susceptibility testing was higher with *Bacillus* spp and *Klebsiella* spp. The molecular technique proved to be more specific and accurate than the convectional technique in identification and characterization of the isolates. As seen from this study, ketogenic diet affirms weight loss and altered the bacterial count.

Keywords: Ketogenic diet, rat gut bacteria, weight, heterotrophic plate count, antibiotics

1. Introduction
Compared to other parts of the body, the human gastrointestinal tract has the highest number of microorganisms, including bacteria, archaea, and eukaryotes [1]. The phrase "gut microbiota" refers to the collection of these bacteria, which is generally accepted to begin at birth [2, 3]. After birth, the gastrointestinal system is soon linked to stressful life events like sickness, antibiotic use, and dietary changes, which results in erratic changes in the microbiota [4]. Antibiotics have a significant negative impact on the variety of the gut microbiota, which can result in the loss of important bacteria and modifications to the host's metabolism. If this takes place, the remaining microorganisms that are resistant to antibiotics will develop and spread [5]. Antibiotic use results in a decrease in bacterial diversity but an increase in the total number of microbes in the gastrointestinal tract. Antibiotic-associated diarrhea, which may result from a pathological overgrowth of Clostridium difficile in the gastrointestinal system after antibiotic treatment, is an example of a recognized consequence. The majority of instances are thought to raise mortality rates for those who have infection with bacteria [6].

When a new-born is delivered vaginally, their microbiota contains a high abundance of lactobacilli for the first few days, reflecting the high load of lactobacilli in the vaginal flora. This suggests that the manner of delivery may also have an impact on the microbiota composition [7]. On the other hand, the microbiota of new-borns delivered via caesarean section is diminished and colonization by the Bacteroides genus is delayed; nonetheless, facultative anaerobes like Clostridium species are still present [8]. While just 41% of new borns delivered via caesarean section have a faecal microbiota that is similar to that of their mothers, 72% of infants delivered vaginally do [9]. Actino bacteria and Proteobacteria, two major phyla that dominate the microbiota formation in the early stages of life, where there is typically little diversity [10]. The intestinal epithelium and the intestinal mucosal barrier that it secretes have co-developed in a way that is tolerant and supportive of the gut microbiota and that also acts as a barrier to pathogenic organisms by the time the gut microbiota has been established and the microbial diversity has increased during the first year of life [11, 12].

Throughout the digestive tract, the human gut microbiota has different microbial compositions. There are acids, oxygen, antimicrobials, and a rapid transit time in the stomach and small intestine [13]. With up to 1012 cells per gram of intestinal content, the colon has the highest known microbial density in comparison [14]. The gut microbiota also includes protozoa, fungi, archaea, and viruses, albeit little is known about their functions [15]. Anaerobes make up over 99% of the bacteria in the gut, however considerable densities of aerobic bacteria can be found in the cecum [16]. Five phyla-Bacteroidetes, Firmicutes, Actinobacteria, Proteobacteria, Verrucomicrobia, and Fusobacteria-dominate the intestinal gut microbiota, with Firmicutes and Bacteroidetes making up 90% of the composition [17]. The gut's Bacteroidetes bacteria are the most Gram-negative, obligate anaerobic, and non-endospore forming [18]. The huge class of Clostridia and the lactic acid bacteria belong to the gram-positive, low-guanine + cytosine-containing Firmicutes. Diet has a direct impact on the makeup of the human microbiota, and as a healthy ecological community is linked to greater health, there are numerous opportunities to improve human health by altering the microbiota through various dietary patterns [19, 20, 21]. The ketogenic diet is a type of eating plan that emphasizes cutting back on carbohydrates and boosting fat intake to induce ketosis [22]. Since 1921, people all over the world have employed the ketogenic diet for its anticonvulsant effects in the treatment of drug-resistant epilepsy. A common treatment option for rapid weight loss at the moment is the ketogenic diet [23]. By reducing the body's sugar stores, this diet works. It will consequently start to digest fat for energy. As a result, the body begins to produce molecules known as ketones, which it uses as fuel. Weight loss can result from the body burning fat. For the health of the host, the gut microbiota performs a number of vital protective, structural, and metabolic tasks. Through a variety of strategies, including direct competition for nutrition, the generation of antimicrobial compounds, and the manipulation of host immune responses, the microbiota's primary job is to prevent pathogen invasion [24]. With particular members that can respond to hormones released by the host, the gut microbiota has the metabolic ability to make and control several chemicals that reach the blood circulation and affect the function of distant organs and systems [25]. The microbiome specifically supplies the host distinct and particular enzymes and metabolic pathways. Many of these metabolic pathways, such as the metabolism of undigested carbohydrates and vitamin production, are advantageous to the host and engaged in either nutrition acquisition or xenobiotic processing [26]. A homeostatic balance between the microorganisms and the host, as well as within microbial communities, is necessary for the preservation of a healthy gut microbiota. Due to an imbalance in the gut microbiota (Dysbiosis), intestinal diseases or disorders might result from failure to maintain this complicated homeostasis (Eubiosis). To create individualized dietary intervention regimens for humans, it is crucial to identify specific dysbiotic profiles during diet therapy.

2. Materials and Methods

2.1 Area of Study, Population and Design

This work was carried out in Pharmacology Department and clinic laboratory, all in Rivers State University, Port Harcourt. This is a pilot study design of albino rat model. Twenty-five female albino rats, weighing 100-122g, were used for this study. They were purchased from the Pharmacology Department, Rivers State University, Port Harcourt, Nigeria. There were five groups with five rats in each, kept in cages under standard conditions and acclimatized for two weeks.

2.2 Ketogenic Food

The standard feed used for this study was Finisher feed (manufactured by TopFeeds Nig. Ltd). The feed and the butter used for this study were also purchased from a local market in Port Harcourt, Nigeria. The ketogenic diet was made according to the standard method of [27]. Then, this method was modified slightly such that another portion of feed consisting 55% of standard feed and 45% of fat was prepared to reflect excessive fat consumption.

2.3 Experiment Design

Twenty five female wistar albino rats were shared into 5 groups, with 5 rats for each group, as follows:

Group 1: was fed with rat feed and water only.

Groups 2 and 3 were placed on ketogenic diet for 7 days

Groups 4 and 5 were fed with ketogenic diet and water for further 14 days.

2.4 Sample Collection Method

After acclimatization, the faeces of each group (1, 2, 3, 4, and 5) were taken aseptically by holding the rat by the neck, face up and pulling the tail downwards. As the stool comes out, it is collected into sterile stool sample tube and taken to the Rivers State University Clinic laboratory for immediate culture. Similarly, at the end of 7 days of ketogenic diet, another stool sample was also collected from each group (1, 2, 3, 4 and 5) the same way by pulling and sent to the same laboratory for culture. At the completion of the 14th day, stool sample were taken as well and sent to the same laboratory for culture.

2.5 Preparation of Media Used and Inoculation

Hektoen Enteric agar, brain Heart Infusion agar, MacConkey, Simmons Citrate Agar, Urea broth and sugars-glucose, lactose, maltose, sucrose, and mannitol. Peptone water was prepared following manufacturer's instructions.

A representative of the bacteria isolates from the mixed culture were sub cultured on freshly prepared sterile MacConkey, Brain Heart Infusion and Hektoen Agar, and incubated overnight at 37 ^{0}C. Brain Heart Infusion Agar was incubated anaerobically for 24 hours as well.

2.6 Heterotrophic Bacteria Count

In a set of 6 test tubes, serial dilution of normal saline was done in the order 1: 10, 1: 100, 1:1000, 1: 10000 and 1: 100000. 10 ml of normal saline was dispensed into the first tube (Original tube), 9 ml into tube 1, 2, 3, 4, and 5. All the tubes were sterilized and left to cool.1 g of the stool sample was weighted into the first tube and mixed as the original sample. Using a sterile pipette, 1 ml was taken from the original sample tube into tube 1 and 1 ml from tube 1 into 2 and so on respectively. From tube 5 1 ml was taken off. As little as 0.1 ml from each tube plated onto different plate count agar (Nutrient agar) with the aid of a glass spreader and incubated for 24 hours. This was done to all the stool samples from the different groups. Colonies on the plates were counted and recorded. The result was calculated using the formula: Number of Colonies × Dilution Factor/0.1. The unit is CFU/g

2.7 Bacteria Susceptibility Testing using Disc Diffusion Method

2.7.1 Preparation of Inoculum

Serial dilution using normal saline in a set of 4 test tubes was done for each isolate. Tube 1 contains 10 ml of normal saline while tube 2, 3, and 4 contains 9 ml of normal saline each. These tubes were sterilized and brought to cool. Using a sterile inoculating wire loop, tube 1 was inoculated with the test organism. 1 ml from tube 1 was dispensed into tube 2, from tube 2 into tube 3, from tube 3 into tube 4 and then, 1 ml taken and discarded. All tubes were incubated overnight at 37 ^{0}C for 24 hours. 0.5 commercially prepared McFarland standard was used to match the turbidity (wavelength 625 nm) of the broths and the tubes that match it are selected for the susceptibility test. Sterile swabs were used to inoculate Mueller - Hinton Agar plate with the test organism and the appropriate antibiotic disk placed the agar plate with the aid of a dispenser. The plates were incubated overnight and diameter zone determined by measuring zones of inhibition with a ruler and results recorded in millimetre (mm).

2.8 Molecular Analysis

2.8.1 Molecular Identification by DNA extraction (Chemical method)

Ten (10) isolates were taken for molecular analysis. The chemical method which involves 3 stages in DNA extraction namely: Lysing, Purification and Precipitation was employed. A ZR fungal/bacterial DNA micro prep extraction kit from Inqaba South Africa was used for the extraction. 750 ul of lysis solution was added to a heavy growth of the pure culture that had been suspended in 200 ul of isotonic buffer in a ZR Bashing Bead Lysis tube. The tube was then placed in a bead beater equipped with a 2 ml tube holder assembly and processed for 5 minutes at maximum speed. Centrifugation was performed on the ZR bashing bead lysis tubes at 10,000 x g for one minute. A Zymo-Spin IIIF spin Filter (orange top) was used to filter 400 ul of the supernatant, which was then centrifuged at 10000 g for one minute. The final volume of 1600 ul of fungal/bacterial DNA binding buffer was added to the filtrates in the collecting tubes. After centrifuging 800 ul at 10,000 x g for 1 minute, the flow through was removed from the collection tube and placed on a Zymo-Spin IIC column. On the same Zymo-spin, the remaining volume was added and spun. After adding 500 ul of fungal/bacterial DNA Wash Buffer to the Zymo-spin IIC in a fresh collection tube and centrifuging at 10,000xg for 1 minute, 200 microliter of the DNA Pre-Was buffer was added. After transferring the Zymo-spin IIC column to a clean 1.5 ul centrifuge tube and adding 100 ul of DNA elution buffer to the column matrix, the DNA was eluted by centrifuging the tube at 10,000 x g for 30 seconds. The extremely pure DNA was then kept at -20 degrees for use in a later reaction.

2.9 Polymerase Chain Reaction

Using the 27F: 5'-AGAGTTTGATCMTGGCTCAG-3 and 1492R: 5'-CGGTTACCTTGTTACGACTT-3 primers on the ABI 9700 Applied Biosystems thermal cycler for 35 cycles, the 16S rRNA region of the rRNA gene of the isolates was amplified. The X2 Dream taq Master mix (taq polymerase, DNTPs, and MgCl) from Inqaba, South Africa, the primers at a concentration of 0.5 uM, and the extracted DNA as template were all ingredients in the PCR mixture. The following were the PCR conditions: Initial denaturation was carried out at 95 °C for 5 minutes, followed by denaturation for 40 seconds at that temperature, annealing at 52 °C, initial extension at 72 °C for 35 cycles, and final extension at 72 °C for 5 minutes. The result was seen on a blue light trans illuminator after being resolved on a 1% agarose gel at 130V for 30 minutes. The result was visualized using a UV trans illuminator after being resolved on a 1% agarose gel at 120V for 15 minutes.

2.10 Sequencing and Phylogenetic Analysis

Inqaba Biotechnology, Pretoria, South Africa, carried out this using a 3510 ABI sequencer and the BigDye Terminator kit. A total volume of 10ul was utilized, with the following components: 2.25ul of 5 x BigDye sequencing buffer, 0.25ul of BigDye® terminator v1.1/v3.1, 10uM Primer PCR primer, and 2–10ng of PCR template per 100bp. There were 32 cycles of 96 °C for 10s, 55 °C for 5s, and 60 °C for 4 minutes in the sequencing conditions. With the help of the bioinformatics algorithm Trace, obtained sequences were modified. BLASTN was used to download related sequences from the National Center for Biotechnology Information (NCBI) database. Using MAFFT, these sequences were aligned. Using MEGA 6.0's Neighbor - Joining approach, the evolutionary history was deduced [28]. The evolutionary history of the taxa examined is assumed to be represented by the bootstrap consensus tree estimated from 500 replicates [29], and their distances were determined using the Jukes-Cantor technique [30].

2.11 Data Analysis

Data were analysed using SPSS version 23. Values were expressed as mean± SD, and presented in tables. Comparison of means with $p \leq 0.05$ was considered statistically significant.

3. Results

3.1 Body Weights of Rats

The weights of rats (in grams) after acclimatization, after seven days and after 14 days for group 1 were 108.00±9.27,

110.20±9.26 and 112.40±14.71. Statistically, there was no difference (p = 0.305) in their body weights. The body weights after acclimatization, seven days of ketogenic diet and fourteen days of ketogenic diet, were respectively 109.40±7.70, 104.40±7.57, and 93.60±11.57 (Group 2), 103.20±3.27, 101.00±3.32, and 95.20±4.09 (Group 3), 111.20±7.98, 108.80±8.19, and 104.20±7.85 (Group 4) and 109.60±6.66, 107.60±6.27 and 102.80±6.38 (Group 5). There was significant difference in 2 (p = 0.049) and 3 (p=0.011). However, there was no significant difference in 4 (p=0.402) and 5 (p=0.268) as shown in the table below.

The table below represents the body weight of rats in all groups after 14 days of acclimatization and after being fed with ketogenic diet for 7 and 14 days.

Table 1: Body Weight in all Groups

	1	2	3	4	5
Rat Weight After Acclimatization (g)	108.00±9.27	109.40±7.70	103.20±3.27	111.20±7.98	109.60±6.66
Rat Weight After 7 Days Treatment (g)	110.20±9.26	104.40±7.57	101.00±3.32	108.80±8.19	107.60±6.27
Rat Weight After Further 14 Days Treatment (g)	112.40±14.71	93.60±11.57	95.20±4.09	104.20±7.85	102.80±6.38
p-value	0.305	0.049	0.011	0.402	0.268
F-value	4.480	3.907	6.672	0.985	1.474

Table 2 shows the susceptibility pattern of the isolates after acclimatization, 7 and 14 days to the antibiotics used. The zone of inhibition was interpreted using the CLSI standard.

Table 2: Antibiotic Resistant Percentage Profile of all Isolates

Antibiotics	*Staphylococcus* spp. (n = 3)	*Klebsiella* spp. (n = 3)	*Bacillus* spp. (n = 3)	*Enterobacter* spp. (n = 3)	*Acinetobacter* spp. (n = 3)
Cefoxitin (30 µg)	0(0%) S	3(100%)	3(100%)	0(0%)	3(100%)
Oxacillin (1 µg)	3(100%) R	3(100%)	3(100%)	3(100%)	3(100%)
Ceftazidime (30 µg)	3(100%) R	0(0%)	3(100%)	0(0%)	3(100%)
Ceftriaxone (30 µg)	0(0%) S	0(0%)	3(100%)	0(0%)	3(100%)
Amoxicillin/clavulanic acid (30 µg)	3(100%) R	3(100%)	3(100%)	3(100%)	3(100%)
Clarithromycin (15 µg)	0(0%) S	0(0%)	3(100%)	3(100%)	3(100%)
Clindamycin (2 µg)	3(100%) R	3(100%)	0(0%)	3(100%)	3(100%)
Gentamycin (30 µg)	0(0%) S	3(100%)	3(100%)	0(0%)	0(0%)
Azithromycin (15 µg)	3(100%) R	3(100%)	0(0%)	3(100%)	0(0%)
Chloramphenicol (30 µg)	3(100%) R	3(100%)	3(100%)	1(33.3%)	2(66.7%)
Sulphamethoxazole/trimethoprim (25 µg)	0(0%) S	3(100%)	3(100%)	3(100%)	0(0%)

Key points: S - sensitivity, R - resistant, n - number of isolates.

Table 3 shows Heterotrophic Bacterial Count after Acclimatization, 7 and 14 Days of Treatment in all Groups. The bacterial count ($x10^5$CFU/ml) after acclimatization, 7 and 14 days are as follows: The counts for group 1 were 108, 111, and 110 respectively, for 2 were 105, 92 and 76 respectively, for 3 were 104, 90 and 78 respectively, for 4 were 101, 95 and 74, and for 5 were 106, 97 and 76. No statistical difference in all.

Table 3: Heterotrophic Bacterial Count after Acclimatization, 7 Days Treatment, and 14 Days Treatment.

	Group 1	2	3	4	5
Bacterial Count After Acclimatization ($x10^5$CFU/ml)	10.8	10.5	10.4	10.1	10.6
Bacterial Count After 7 Days Treatment ($x10^5$CFU/ml)	11.1	92	90	95	97
Bacterial Count After Further 14 Days Treatment ($x10^5$CFU/ml)	11.0	76	78	74	76
p-value	0.979	0.98	0.154	0.107	0.078
X^2-value	0.043	4.637	3.735	4.467	5.097

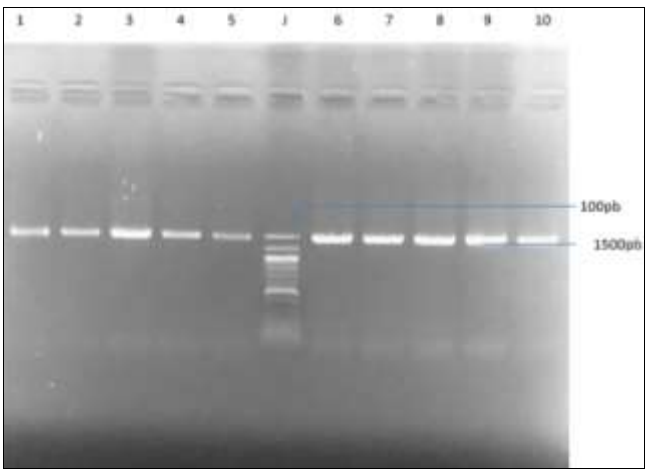

Plate 1: Represent bacterial isolates with Lane 1 – 10 showing 16SrRNA gene bands (1500bp) and Lane J showing the 100bp DNA ladder

Using the formula

$$\text{The Unit: CFU/ml.} = \frac{\text{Number of Colonies} \times \text{Dilution Factor}}{0.1 \text{ (chosen aliquot)}}$$

Plate 1 represents bacterial isolates where Lane 1 – 10 represent 16SrRNA gene bands (1500bp) and Lane J 100bp DNA ladder.

3.2 Phylogenetic Analysis

The outcome of the mega blast search for extremely similar sequences from the NCBI non-redundant nucleotide (nr/nt) database gave an exact match. The isolates' 16S rRNA revealed a percentage like that of other species. The evolutionary distances computed using the Jukes – Cantor method were in agreement with the phylogenetic placement of the 16S rRNA of the isolates within the *Bacillus, Staphylococcus, Klebsiella, Acinetobacter, Comamonas,* and *Enterobacter* species and revealed a closely relatedness *to Bacillus cereus, Bacillus thuringiensis, Bacillus pumilus, Staphylococcus arlettae, Klebsiella variicola, Acinetobacter nosocomialis, Comamonas testosteroni, Enterobacter cancerogenus* and *Enterobacter cloacae* as seen in Figure 1.

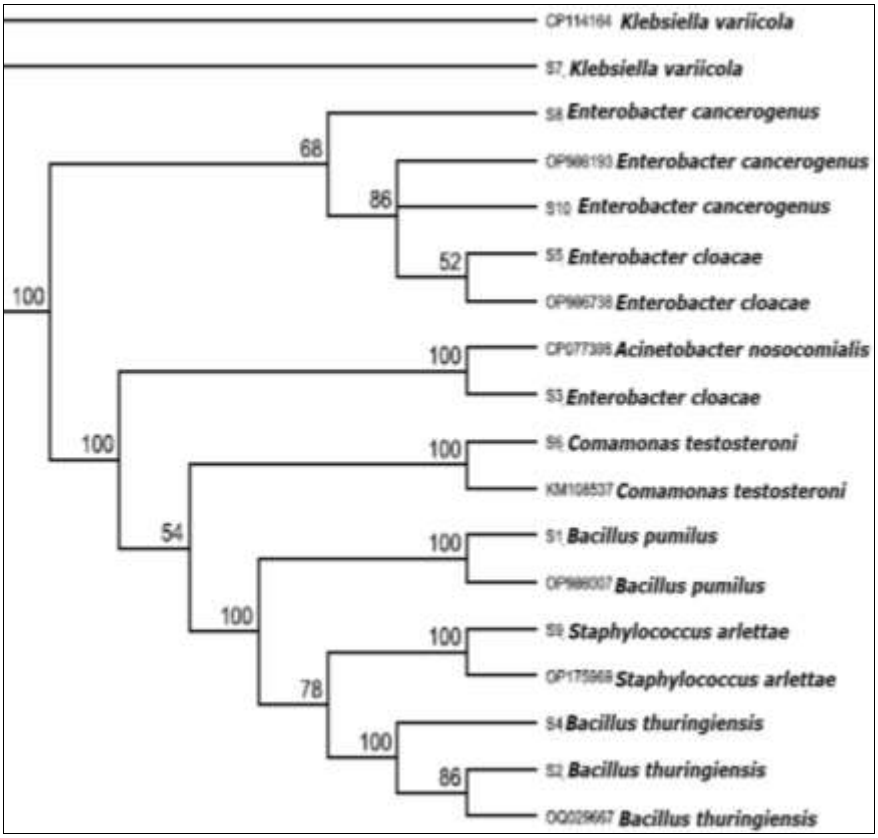

Fig 1: Phylogenetic Tree showing the Evolutionary Distance between the Bacterial Isolates

4. Discussion

This study evaluated the effect of ketogenic diet in albino rat gut microbiota. The body weight of rats after acclimatization, after 7 and 14 days for group 1 (control) were 108.00±9.27, 110.20±9.26 and 112.40±14.71 respectively. There was weight gain in the rats. The weight of rats after acclimatization, after 7 and after 14 days of diet, were 109.40±7.70, 104.40±7.57, and 93.60±11.57 (Group 2), 103.20±3.27, 101.00±3.32, and 95.20±4.09 (Group 3), 111.20±7.98, 108.80±8.19, and 104.20±7.85 (Group 4) and 109.60±6.66, 107.60±6.

27 and 102.80±6.38 (Group 5). There is significant weight loss in group 2 and 3 following a cut off of $p< 0.05$. This implies that the ketogenic diet may have impact on weight reduction in the rats. This could probably be due to fat metabolism. This is consistent with the findings of [31], who reported that a ketogenic diet can cause a metabolic shift in fat breakdown that can later decrease hunger while producing ketone bodies, and the findings of [36], who states that a ketogenic diet may shift the body to a state of ketogenesis through fat breakdown that may change the gut microbiota. The microbiota may help with weight loss caused by a ketogenic diet, according to [37]. The body weight of the rats in groups 4 and 5 that were on the ketogenic diet for an additional 14 days did not significantly alter. The fat may not have been metabolized, in this case. This supports the findings of [38], who suggested that weight reduction may not always be necessary because a different study in mice revealed no further weight loss after 22 weeks of adhering to a ketogenic diet. This demonstrates a possible distinction between a ketogenic diet's short- and long-term effects on body weight.

The heterotrophic plate count of bacteria revealed a decrease in the number of counts following high fat diet for 7 days and 14 days. The bacterial counts from group 1 after acclimatization, 7 days and 14 days of normal diet maintained a relatively stable rate while group 2, 3, 4 and 5 showed decrease in bacterial count following ketogenic diet for 7 days and 14 days. The bacteria count in all the groups after acclimatization is higher than the counts in group 2, 3, 4 and 5 that were fed with ketogenic diet. Hence, there is decrease in count. This implies that ketogenic diet probably

had impact on the gut microbiota. This can be due to a change in diet. Similar findings were reported by [39], who also suggests that modifying one's diet can alter the quantity of bacteria present. This result is also consistent with a previous study by which fed healthy mice a ketogenic diet and found that it reduced the number of bacteria cells. In terms of statistics, there was no discernible variation in the number of bacteria in any of the groups. From this investigation, it was discovered that the ketogenic diet-fed rats had smaller stools. Constipation may be the cause of this. Also, noted that, as compared to a high-carb diet, a ketogenic diet negatively impacted bowel health by reducing stool mass, bowel movement frequency, and faecal butyrate concentration.

The susceptibility testing of all the isolated organisms showed that *Staphylococcus* species were sensitive to cefoxitin, ceftriaxone, clarithromycin, gentamycin, and sulphamethoxazole/trimethoprim but resistant to Oxacillin, ceftazidime, clindamycin, Amoxycillin/Clavulanic Acid, azithromycin and chloramphenicol. *Klebsiella* species were sensitive to ceftazidime, ceftriaxone and clarithromycin but resistant to the rest antibiotics. *Bacillus* species were sensitive to only clindamycin and azithromycin and resistant to the rest antibiotics. *Enterobacter* species were sensitive to cefoxitin, ceftazidime, ceftriaxone, Amoxycillin/Clavulanic Acid, clindamycin and chloramphenicol and resistant to the rest antibiotics. *Acinetobacter species* were sensitive to clindamycin, gentamycin, azithromycin, chloramphenicol and sulphamethoxazole/trimethoprim and resistant to the rest antibiotics. *Bacillus* and *Acinetobacter* species were resistant to cefoxitin, oxacillin, ceftazidime, ceftriaxone, Amoxycillin/Clavulanic Acid, and clarithromycin. Using the CLSI standard in interpreting the zone of diameter of inhibition, the isolates showed higher degree of resistance with *Bacillus* being the most resistant organism.

The evolutionary distances calculated using the Jukes-Cantor technique for the isolates' molecular identification were similar to the phylogenetic placement of the isolates' 16S rRNA within the *Bacillus, Staphylococcus, Klebsiella, Acinetobacter, Comamonas,* and *Enterobacter* species and revealed a close relatedness to S4 *Bacillus thuringiensis, S2 Bacillus thuringiensis,* S1 *Bacillus pumilus,* OP98600 *Bacillus pumilus,* S9 *Staphylococcus arlettae,*OP175968 *Staphylococcus arlettae,* CP114164 *Klebsiella variicola,* S7 *Klebsiella variicola,* CP077398 *Acinetobacter nosocomialis,* S6 *Comamonas testosteroni,* KM108537 *Comamonas testoeroni*, S8 *Enterobacter cancerogenus, OP986193 Enterobacter cancerogenus,* S10 *Enterobacter cancerogenus,* S5 *Enterobacter cloacae* and OP986738 *Enterobacter cloacae*. This shows similarities with [45] report who also identified similar organisms from rat gut.

5. Conclusion

From this study, the effect of ketogenic diet was observed in all the groups that were fed with ketogenic diet. There was weight loss in the groups that were fed with ketogenic nutrition for 7 days and 14 days. The group 1 (control) that was fed with normal diet did not show weight lost throughout the study. Comparatively, there was significant change in weight in group 2 and 3 but no significant difference in group 4 and 5. Additionally, the heterotrophic plate count in the control group was relatively stable while there was decrease in total bacteria count in rats fed with ketogenic diet. Also, group 4 and 5 had the lowest percentage of bacterial count. There was decrease in the bacterial count in all the groups. Reduced stool mass was also observed in the rats that were fed with ketogenic diet. Using the CLSI standard in interpreting the zone diameter of inhibition, the isolates showed higher degree of resistance to the antibiotics used in this study, with *Bacillus* being the most resistant organism. The molecular analysis proved to be more specific and accurate than the convectional technique.

6. Conflict of Interest

Not available

7. Financial Support

Not available

8. References

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