Molecular Characterisation of Gut Bacteria in Wista Rat after Green Tea Consumption

ABSTRACT

Green tea can influence the gut bacteria by either stimulating or hindering the growth of specific species. This experimental study was carried out in Rivers State , Nigeria to molecularly characterize gut bacteria in Wista rats after green tea consumption.. Twenty-five (25) rats divided into five groups were used ; each group had five (5) rats having an average weight range of 70g – 130g. After acclimatization of 2 weeks, groups (2, 3, 4 & 5) were feed with green tea extract based on standard method using oral gavage with rat feed pellet and water for seven (7) days and fourteen (14) days respectively based on their body weights and group 1 (control) was fed with rat feed pellet and water only for same duration and their weights recorded. Stool samples were collected aseptically by pulling from each group into sterile stool sample bottles and sent to the laboratory for immediate analysis. Conventional microbiological methods of culturing and molecular methods were utilized for identification of isolates, total heterotrophic plate count and antibiotic susceptibility were also done. The data from this study were analysed using SPSS version 23, to survey difference in gut bacterial count of wista rats after fed with green tea, rat weight across the study groups, and the values were expressed as mean ± SD at a 5% alpha level.
The study reported a statistical difference in bacterial plate count at ($P \leq 0.05$) in Groups 2, 3, 4, and 5 after 14 Days of Acclimatization, after 7 days of Treatment and after further 14 Days of Treatment. Group 1 showed no statistical significance at $P=0.212$. Furthermore, there was significant difference in weight comparison at ($P \leq 0.05$) in rats contained in all groupsafter 7 days and after further 14 days of treatment, rats in group 1 showed weight gain. There was weight loss in all the treatment groups (2 ,3, 4, and 5). Antibiotic-resistant assay pattern of the isolates showed multiple drugs resistance to all the antibiotics used with *Bacillus* and *Enterobacter* being the most resistant isolates. The molecular characterization and identification of the isolates revealed close relatedness to *Staphylococcusarlettae*, *Enterobactercloacae*, *Enterobactercancerogenus*, *Acinetobacternosocomialis*, *Comamonastestosterone*, *Klebsiellaveriicola*, *Bacillusthuringiensis*, *Bacilluspumilus* and *Bacillus cereus*, it showed that the molecular technique proved to be morespecific and accurate than the convectional technique in the identification and characterization of theisolates. Also, the gut bacterial count of the rats that were treated with the tea were significantly raised indicating that the tea has the potential to influence the abundance and diversity of gut bacteria. As seen in this study, molecular characterisation of gut bacteria identifies bacteria upto their specie level and they are influenced by diet.

Keywords: Molecular characterisation, rat gut bacteria, heterotrophic plate count, antibiotics

1. Introduction

The human gastrointestinal tract (GI) also known as the gut is home to a complex and dynamic community of bacteria known as the gut bacteria, which has a significant impact on the host during homeostasis and disease. Several factors contribute to the development of the human gut bacteria during childhood. Diet is thought to be one of the most important factors in shaping the gut bacteria throughout life. The gut bacteria has co-evolved with the host over thousands of years to form an intricate and mutually beneficial relationship [1]. The number of microorganisms inhabiting the GI tract has been estimated to be greater than 10^{14}, which includes 10 times more bacterial cells than human cells and more than 100 times the amount of genomic content (microbiome) as the human genome [2]. However, a recently revised estimate suggests that the human bacterial cell ratio is closer to 1:1 [3]. The gut bacteria provides numerous benefits to the host by performing a variety of physiological functions such as strengthening gut integrity or shaping the intestinal epithelium, harvesting energy, protecting against pathogens, and regulating host immunity [4]. However, these mechanisms may be disrupted as a result of altered microbial composition, a condition known as dysbiosis. The pathogenesis of many inflammatory diseases and infections has been linked to altered gut bacterial composition (dysbiosis). With the development of increasingly sophisticated methods for profiling and characterising complex ecosystems, the gut bacteria's role in a wide range of intestinal and extra-intestinal diseases has become increasingly clear [5].

Green tea is a beverage that has been consumed for thousands of years and has not lost popularity due to various health benefits, pleasantness or social characteristics [6]. It is made from the infusion of *Camellia sinensis* plant leaves and has been used in traditional Chinese medicine for over 3000 years. Over the centuries, it has spread from China to the rest of Asia, then to Europe, America, and finally Africa. This growth was made possible by colonization and

various trade routes such as the Silk Road. As a result, green tea is one of the most popular beverages in the world, and its popularity has recently increased as a result of extensive research claiming associations between green tea and health [7].

To make green tea, the leaves are subjected to various processes such as high-temperature steaming or roasting, which causes the oxidative enzyme polyphenol oxidase to be inactivated. The inactivation of polyphenol oxidase prevents the majority of its polyphenolic compounds (catechins, flavones, anthocyanins, and phenolic acids and some other minor polyphenols that also exist such as epigallocatechin gallate (EGCG), flavanol glycoside, and tannins) from being oxidized [8]. Its antioxidant and anti-inflammatory properties has been linked to a variety of health benefits including the treatment of obesity, diabetes, cancer, kidney, liver, brain, and bone diseases, among others. Some of these health benefits on the other hand, have been attributed to an interaction between the tea's bioactive compounds and the gut bacteria [9]. Its polyphenols have low bioavailability, as has been extensively demonstrated over the last decade, with the majority of them reaching the large intestine and being metabolized by gut bacteria. The health benefits of tea-bacteria interaction could be a direct effect of microbial polyphenol metabolites or an indirect effect resulting from stimulation of specific beneficial gut bacteria [10]. At the same time, gut bacteria is linked to host health and plays an important role in chronic diseases such as obesity, diabetes, inflammatory bowel disease, and even neurological disorders. As a result, considerable effort is being expended in attempting to comprehend how the gut bacteria specifically functions, particularly in relation to diet,[11]. . Green tea can promote the growth of beneficial bacteria, inhibit the growth of harmful bacteria, or increase the production of beneficial metabolites like short chain fatty acids. Green tea can influence gut inflammatory processes, colorectal cancer, redox processes in the intestine, energy scavenging, macronutrient metabolism

by gut bacteria, and obesity by shaping and modulating the gut bacteria. A number of chronic diseases have been linked to oxidative stress. Green tea polyphenols, in particular, are thought to be powerful antioxidants and free radical scavengers. As a result, they are thought to play a protective role against oxidative stress-related chronic diseases. This study was done to carry out the molecular characterization of gut bacteria of wistar rats after green tea consumption.

2. Materials and Methods

2.1 Area of Study, Population and Design

This study was done in the Department of Anatomy and Clinical Laboratory Rivers State University, Port Harcourt, Nigeria, all in the same University. It is a pilot study design of wista rat model. Twenty-five(25) Wistar rats, procured from Rivers State University, Pharmacology Department were transferred to Department of Human Anatomy Rivers State University, and confined at the animal house in a room at a temperature of between 35^{oC} and $37^{o}C$ were used for this study. There were five groups with five rats in each group, kept in standard cages under good laboratory conditions and acclimatized for two weeks.

2.2 Green Tea

The green tea utilized for this study was procured from University of Port- Harcourt Everyday Emporium Grocery Shop Business District Choba. It was ensured that the label-seal was not broken as well as the expiration date (June, 2027) intact. The green tea was made based on standard method [12] of preparation by infusing 1 tea bag in 148mls of warm water.

2.3 Growers Rat Feed Pellet

The convectional rat feed used for this study was growers pellet (manufactured by Top Feeds Nig. Ltd). The feed used for this study was purchased from a local market in Port Harcourt,

Nigeria. First ethics in animal handling and care was observed while utilizing the rat feed all through the study.

2.4 Experiment Design

Twenty five wistar rats were shared into 5 groups, with 5 rats for each group, as follows: Group 1: was fed with rat feed and water only all through the study. Groups 2, 3, 4, and 5 were fed with rat feed, water and green tea for 7 and 14 days respectively.

2.5 Sample Collection Method

After acclimatization and weight of rats recorded, the faeces of each group (1, 2, 3, 4, and 5) were aseptically collected and pulled by holding the rat on the soft fold of the neck between the ears facing up. Pulling the tail backwards caused pressure that made stool to be passed. Stool was collected into sterile, universal stool sample bottles, properly labelled and taken to the Rivers State University Clinic laboratory for immediate culture. Similarly, by the end of 7^{th} day and 14^{th} day administration of green tea to treatment groups 2, 3, 4 and 5, the rats were also weighed and recorded, another stool sample was collected from each treatment group as well as group 1 (Control) the same way by pulling and immediately sent to the same laboratory for immediate culture.

2.6 Preparation of Media Used and Inoculation

Hektoen Enteric agar, brain Heart Infusion agar, MacConkey, Simmons Citrate Agar, Urea broth, Peptone water and sugars - glucose, lactose, maltose, sucrose, and mannitol were 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 0C. Brain Heart Infusion Agar was incubated anaerobically for 24 hours as well.

2.7 Heterotrophic Bacteria Count

Serial dilution of normal saline was done in a set of 5 test tubes in the order ^{1} /10, ^{1}/100, ^{1}/1000, ^{1}/ 10000 and ^{1}/ 100000. The original tube (Stock tube) had 10 ml of normal saline, while 9 ml was dispensed into tubes 1, 2, 3, 4, and 5. All the tubes were sterilized and left to cool at room temperature. One (1) g of the rat stool sample was weighted out and transferred into the original tube and mixed as the original sample. With the aid of a sterile pipette, 1 ml was collected from the original sample tube into tube 1 and 1 ml from tube 1 into tube 2 and so on respectively. One (1) ml was taken off from tube 5, as little as 0.1 ml from each tube was inoculated onto Nutrient agar with the aid of a glass spreader and was incubated for 24 hours at 37^{o}C. This procedure was done to all the stool samples from the different groups. Colonies on the plates were counted and recorded. Thereafter, the result was calculated using the formula: Number of Colonies × Dilution Factor/0.1, the unit is CFU/ml.

2.8 Bacteria Susceptibility Testing

Bacteria susceptibility test was done using disc diffusion method and inoculum was prepared in a set of 4 test tubes. Serial dilution using normal saline was done for each isolate. Tube 1 contains 10 ml of normal saline while tubes 2, 3, and 4 contains 9 ml of normal saline each. These tubes were sterilized and brought to cool at room temperature. With the aid of a sterile inoculating wire loop, the test bacteria was transferred to tube 1. Then, 1ml from tube 1 was dispensed into tube 2, from tube 2 into tube 3, from tube 3 into tube 4 and then, 1 ml was taken and discarded.

All tubes were aerobically incubated overnight at 37 ^{o}C for 24 hours. Commercially prepared 0.5 McFarland standard was used to match the turbidity (wavelength 625 nm) of the broths and the tubes that match it were selected for susceptibility test. Sterile swabs were used to inoculate

Mueller - Hinton Agar plate with the test organism and the appropriate antibiotic disk placed on the agar plate with the aid of a dispenser. The plates were incubated overnight and zones of diameter determined by measuring zones of inhibition with a transparent ruler and results recorded in millimetre (mm).

2.9 Molecular Analysis

2.9.1 Molecular Identification by DNA extraction

Ten (10) isolates were collected for molecular analysis. The chemical approach was used for DNA extraction, which consists of three stages: lysing, purification, and precipitation. The extraction was performed using an Inqaba South Africa ZR fungal/bacterial DNA micro prep extraction kit. In a ZR Bashing Bead Lysis tube, 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. The tube was then placed in a bead beater fitted with a 2 ml tube holder assembly and treated for 5 minutes at maximum speed. The ZR bashing bead lysis tubes were centrifuged at 10,000 x g for one minute. The supernatant 400ul was filtered using a Zymo-Spin IIIF spin Filter (orange top) and centrifuged at 10000 g for one minute. The filtrates in the collecting tubes were treated with 1600 ul of fungal/bacterial DNA binding buffer. The flow through was taken from the collecting tube and deposited on a Zymo-Spin IIC column after centrifuging 800 ul at 10,000 x g for 1 minute. The flow through was extracted from the collection tube and deposited on a Zymo-Spin IIC column. The remaining volume was added and spun on the same Zymo-spin. After adding 500 ul of fungal/bacterial DNA Wash Buffer to a new collection tube and centrifuging at 10,000xg for 1 minute, 200 microliter of DNA Pre-Wash buffer was added. The DNA was eluted by centrifuging the tube at 10,000 x g for 30 seconds 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 very pure DNA was then stored at -20 degrees for use in a later experiment.

2.9.2 Polymerase Chain Reaction

The isolates 16S rRNA gene was amplified using the 27F: 5'-AGAGTTTGATCMTGGCTCAG-3 and 1492R: 5'-CGGTTACCTTGTTACGACTT-3 primers on the ABI 9700 Applied Biosystems thermal cycler for 35 cycles. The PCR mixture included the X2 Dream taq Master mix (taq polymerase, DNTPs, and MgCl) from Inqaba, South Africa, the primers at 0.5 uM concentration, and the extracted DNA as template. The following were the PCR conditions: Initial denaturation was performed 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. After being resolved on a 1% agarose gel at 130V for 30 minutes, the result was observed on a blue light trans illuminator. After being resolved on a 1% agarose gel at 120V for 15 minutes, the result was visualised with a UV trans illuminator.

2.9.3 Sequencing and Phylogenetic Analysis

This was done by Inqaba Biotechnology in Pretoria, South Africa, utilising a 3510 ABI sequencer and the BigDye Terminator kit. The following components were used in a total volume of 10ul: 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 were used. In the sequencing conditions, there were 32 cycles of 96 °C for 10s, 55 °C for 5s, and 60 °C for 4 minutes.

The retrieved sequences were changed using the bioinformatics method Trace. The BLASTN programme was used to retrieve similar sequences from the National Centre for Biotechnology

Information (NCBI) database. These sequences were aligned using MAFFT. The evolutionary history was determined using MEGA 6.0's Neighbour - Joining method . The bootstrap consensus tree produced from 500 iterations is believed to reflect the evolutionary history of the taxa studied, and their distances were calculated using the Jukes-Cantor technique.

2.10 Data Analysis

SPSS version 23 was used to examine the data. Tables were used to present the values, which were given as mean ±SD and presented in tables. Mean comparisons with $p \leq 0.05$ were considered statistically significant.

3. Results

3.1 Body Weight of Rats

The weight of rats (in grams) after acclimatisation, seven days, and further fourteen days for Group 1 were 102.00 ± 3.39, 107.80 ± 3.56 and 112.00 ± 2.35 respectively. There was a significant difference ($p<0.001$) in their body weight. The body weights after acclimatisation, seven days of green tea, and further fourteen days of green tea administration were respectively 105.80 ± 3.35, 103.80 ± 1.48 and 95.60 ± 4.28 for Group 2; 104. 40 ± 4.04, 100 .40 ± 4.04 and 96.00 ± 3.69 for Group 3; 107.20 ± 7.46, 101.20 ± 6.69 and 89.60 ± 7.60 for Group 4; 105.20 ± 5.81, 99.00 ± 5.96 and 91.00 ± 6.44 for Group 5. There was a significant difference in Group 2 ($p<0.001$). There was a significant difference in Group 3 ($p=0.018$).There was a significant difference in Group 4 ($p=0.008$). There was also a significant difference in Group 5 ($p=0.010$) as illustrated in the table below. The table below shows the body weight of rats in all groups after acclimatisation, 7 and 14 days of green tea administration.

Table 1: Body Weight in all Groups

Groups	**1**	**2**	**3**	**4**	**5**
Rat Weight After Acclimatization (g)	102.00 ± 3.39	105.80 ± 3.35	104. 40 ± 4.04	107.20 ± 7.46	105.20 ± 5.81
Rat Weight After 7 Days Treatment (g)	107.80 ± 3.56	103.80 ± 1.48	100 .40 ± 4.04	101.20 ± 6.69	99.00 ± 5.96
Rat Weight After Further 14 Days Treatment (g)	112.00 ± 2.35	95.60 ± 4.28	96.00 ± 3.69	89.60 ± 7.60	91.00 ± 6.44
P- value	0.001	0.001	0.018	0.008	0.010
F - value	12.734	13.823	5.744	7.358	6.867

Table 2 shows the Heterotrophic Bacterial Count in all Groups after Acclimatisation, 7 days and further 14 Days of green tea administration.

The bacterial count ($x10^5$CFU/ml) after acclimatisation, 7 days and further 14 days are as follows:

The counts for Group 1 were: 130, 132, and 131 respectively; for Group 2 were: 129, 127 and 190 respectively; for Group 3 were: 128, 126 and 980 respectively ; for Group 4 were : 129, 115, and 890 respectively and for Group 5 were: 130, 110, and 860 respectively. There was no significant difference in group 1. Notably, there was significant difference in Groups (2, 3, 4 and 5).

Table 2: Heterotrophic Bacterial Count after Acclimatization, 7 Days Treatment, and after further 14 Days Treatment.

Groups	**1**	**2**	**3**	**4**	**5**
Bacterial Count After Acclimatisation (x10^5CFU/ml)	13.0	12.9	12.8	12.9	13.0
Bacterial Count After 7 Days Treatment (x10^5CFU/ml)	13.2	12.7	12.6	11.5	11.0
Bacterial Count After Further 14 Days Treatment (x10^5CFU/ml)	13.1	19.0	98.0	89.0	86.0
P – value	0.212	0.015	0.001	0.001	0.002
X^2-value	15.427	10.667	23.383	5.876	11.792

Table3showsthesusceptibilitypatternoftheisolatesafteracclimatization, 7and14daystotheantibioticsused.The zoneofinhibition wasinterpreted usingtheCLSIstandard.

Table 3 : Antibiotic Resistant Percentage Profile of all Isolates

ANTIBIOTICS		ISOLATES				
		Acinetobacter spp	*Bacillus spp*	*Enterobacter spp*	*Klebsiella spp*	*Staphylococcus spp*
Amoxycillin\ Clavulanic Acid	30µg	R	R	R	R	R
Azythromycin	15µg	S	S	S	S	S
Chloramphenicol	30µg	R	S	S	S	S
Ceftazidime	30µg	R	R	R	R	R
Clarithromycin	15µg	S	S	S	S	S
Ceftriazone	30µg	R	R	R	R	R
Clindamycin	2µg	S	R	R	R	R
Cefoxitin	30µg	R	R	R	R	R
Gentamycin	30µg	S	R	R	S	S
Oxacillin	1µg	R	R	R	R	R
Sulphamethoxazole\ Trimethoprim	25µg	R	R	R	R	R

Key points: S – sensitivity, R - resistant

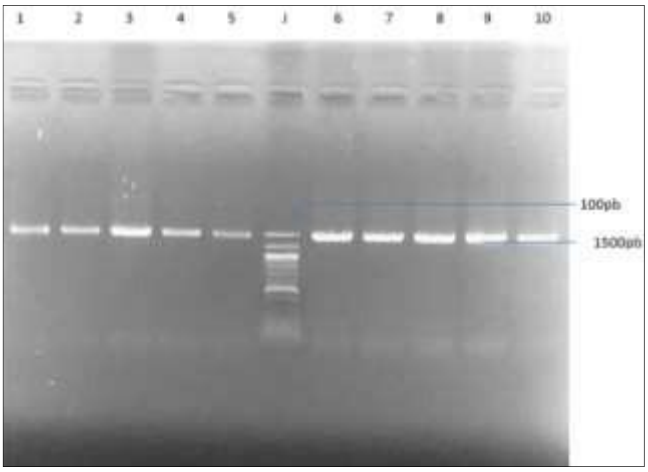

Plate 1: Represents bacterial isolates with Lanes 1 – 10 showing 16SrRNA gene bands (1500bp) and Lane J showing the 100bp DNA ladder

Plate 1 represent bacterial isolates while lanes Lane 1 – 10 shows 16SrRNA gene bands (1500bp) and Lane J show the 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 *Acinetobacter, Bacillus, Comamonas, Enterobacter, Klebsiella* and *Staphylococcus* species and revealed a closely relatedness to S4 *Bacillus thuringiensis, S2Bacillusthuringiensis,*S1*Bacilluspumilus,*OP98600*Bacilluspumilus,*S9*Staphylococcusarlettae,*OP175968*Staphylococcus arlettae,* CP114164 *Klebsiella variicola,* S7*Klebsiella variicola,* CP077398 *Acinetobacter nosocomialis,*S6*Comamonastestosteroni,*KM108537*Comamonastestoeroni,*S8*Enterobactercan*

*cerogenus,OP986193Enterobactercancerogenus,*S10*Enterobactercancerogenus,*S5*Enterobact ercloacae*andOP986738*Enterobactercloacae,* as seen in figure 1 below.

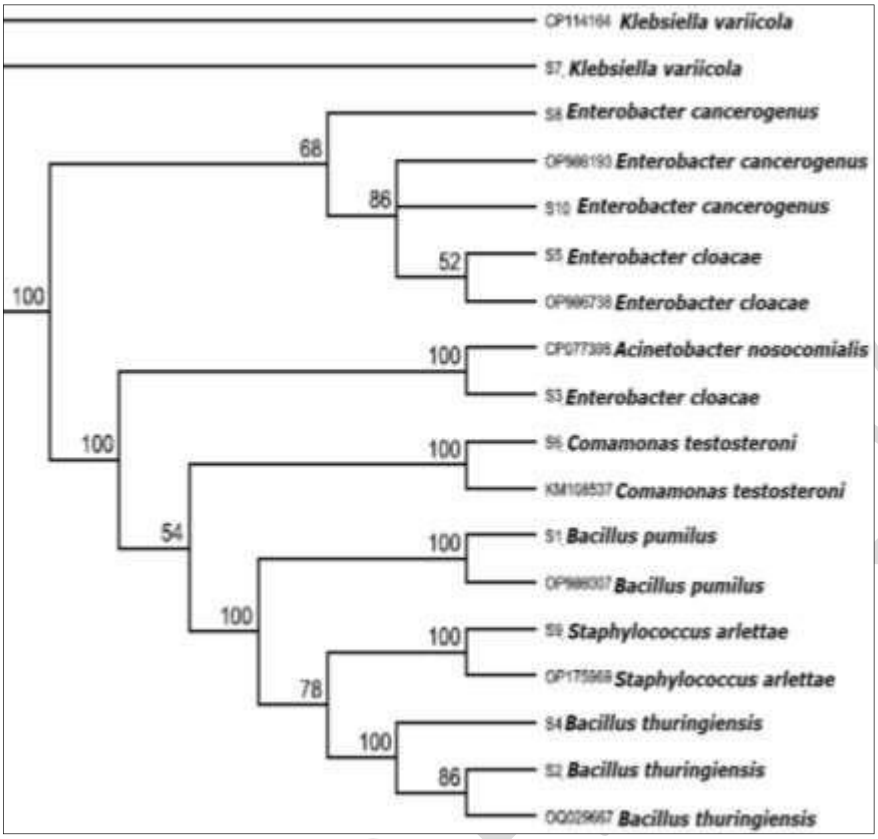

Figure 1: Phylogenetic Tree showing the Evolutionary Distance between the Bacterial Isolates

4. Discussion

This research shows the effect of green tea on the gut bacteria in wistar rats [13]. This study's discovery of the phytonutrient components of green tea was similar to the work of [14]

Herbal teas have been found to include high levels of phytonutrients, which are responsible for their beneficial effects [15] and gut bacteria modulation [16].

These results agree with that of [17]. These tea constituents have been reported to have antimicrobial, anti-inflammatory, anti-carcinogenic, anti-oxidative, anti-arthritic, neuroprotective,

and cholesterol-lowering properties, making them an effective preventive agent against infections [18], and can also have effects on gut bacteria [19]. This findings is consistent with the work of [18]. The body weight of rats after acclimatization, after 7 days and further 14 days of green tea administration for group 1 (control) were 102.00 ± 3.39, 107.80 ± 3.56 and 112.00 ± 2.35 respectively, there was weight gain in the rats. The results from this study indicate significant increase as indicated above in the body weight of the control as the days increased, until the end of the study. This may be due to the fact that they had normal diet during the study. On the contrary, the rats in the treatment groups had significant decreases in their weight as the study progressed thus: the weight of rats after acclimatization, after 7 days and after further 14 days of green tea administration, were 105.80 ± 3.35, 103.80 ± 1.48 , and 95.60 ± 4.28 respectively in Group 2 ; 104. 40 ± 4.04, 100. 40 ± 4.04, and 96.00 ± 3.69 respectively in Group 3; 107.20 ± 7.46, 101.20 ± 6.69 and 89.60 ± 7.60 respectively in Group 4 and105.20 ± 5.81, 99.00 ± 5.96 and 91.00 ± 6.44 in Group 5 respectively. There was significant weight loss in groups 2, 3, 4 and 5 following green tea administration, this implies that the green tea used in this study may have impact on weight reduction in the rats [20]. This observation could be due to the presence of phytonutrients, which are reported to have the ability to promote weight loss by promoting thermogenesis and fat oxidation [21]. Phytonutrients have the potential to increase lipid excretion thereby increasing the faecal fatty acid content, thereby preventing high-fat diet–induced obesity. This finding agrees with the works of [22, 23, 24] who stated in their work that green tea has cholesterol-lowering effect in rats administered with various doses of tea. Thus, the green tea used in this study has the potential to be used in weight reduction therapies, with the dosage and duration used in this study.

In the study of [25] green tea attenuated the increasing tendency of the gut bacteria ratio caused by the High fat diet in both Cure and Prevent experiments, suggesting that a reduction in the ratio by green tea consumption depended on the physiological state of rat is in contrary with this study which showed statistical significance in bacteria count in all the treatment groups administered with green tea at different doses across the study period. Interestingly, this study report compared the weight loss effect of green tea with rats fed on growers rat pellet, and found that green tea over time and on increased dose has anti-obesity effect. The result of this present study which revealed significant difference in weight comparison, is in opposition with that of [26].

Findings from this study indicated that the control group showed no significant change in bacterial plate count, while there was significant difference in the gut bacterial plate count of the treated rats and this was dependent on the duration. This is in tandem with the results of [27] who worked on the antimicrobial properties of green tea in their research. Groups 2, 3, 4, and 5 showed significant decrease in bacteria plate count after acclimatization and 7 days after treatment. However, after further 14 days treatment there was significant increase in plate count throughout these groups respectively. This study is similar to that of [28] that indicated the increase of gut bacterial growths in rats. From this study, it was discovered that the rats fed with green tea had free bowel movement. Hence, orchestrated semi-formed loose stool.

In further consideration of this present study, the susceptibility testing of all the isolated bacteria showed that *Acinetobacter* species were sensitive to Azythromycin, Clarithromycin, Clindamycin and Gentamycin but resistant to Amoxycillin/Clavulanic Acid, Chloramphenicol, Ceftazidime, Ceftriazone, Cefoxitin, Oxacillin and Sulphamethoxazole/Trimethoprim. *Bacillus* species were sensitive to Azythromycin, Chloramphenicol, Clarithromycin but resistant to

Amoxycillin/Clavulanic Acid, Ceftazidime, Ceftriazone, Clindamycin, Cefoxitin, Gentamycin, Oxacillin and Sulphamethoxazole/Trimethoprim. *Enterobacter* species were sensitive to Azythromycin, Chloramphenicol and Clarithromycin but resistant to Amoxycillin /Clavulanic Acid, Ceftazidime, Ceftriazone, Clindamycin, Cefoxitin, Gentamycin, Oxacillin and Sulphamethozaxole/Trimethoprinprin. *Klebsiella* species were sensitive to Azythromycin, Chloramphenicol, Clarithromycin and Gentamycin but resistant to Amoxycillin /Clavulanic Acid, Ceftazidime, Ceftriazone, Clindamycin, Cefoxitin, Oxacillin and Sulphamethozaxole/Trimethoprinprin. *Staphylococcus* species were sensitive to Azythromycin, Chloramphenicol, Clarithromycin and Gentamycin but resistant to Amoxycillin /Clavulanic Acid, Ceftazidime, Ceftriazone, Clindamycin, Cefoxitin, Oxacillin and Sulphamethozaxole/Trimethoprinprin. The CLSI standard was used for interpreting the zone of diameter inhibition; the isolates in this study demonstrated multiple drug resistance with *Bacillus* and *Enterobacter* being the most resistant bacteria. This multi - drug resistant is consistent with the findings of [29, 30, 31], who reported on the bidirectional effect of antibiotics on gut bacteria.

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 *Acinetobacter*, *Bacillus, Comamonas, Enterobacter, Klebsiella and Staphylococcus* species and also revealed a close relatedness to S4 Bacillus thuringiensis, S2 Bacillus thuringiensis, S1 *Bacilluspumilus,* OP98600 *Bacillus pumilus*, S9 *Staphylococcus arlettae*, OP175968 *Staphylococcusarlettae,* CP114164 *Klebsiella variicola*, S7 *Klebsiella variicola*, CP077398 *Acinetobacter nosocomialis,* S6 *Comamonas testosteroni,* KM108537 *Comamonas testosteroni,* S8 *Enterobacter cancerogenus,* OP986193 *Enterobacter cancerogenus,* S10

Enterobacter cancerogenus, S5 *Enterobacter cloacae* and OP986738 *Enterobacter cloacae* this findings is in agreement with the work of [32].

5. Conclusion

Interestingly, this study report contrasted the weight loss effect of green tea with rats fed on regular rat pellet; group 1 (control), and discovered that green tea has an anti-obesity effect on groups fed with green tea over time and at greater doses. As a result, the weight of rats fed with green tea decreased significantly during the trial, showing that the tea can be utilised for weight management.

Furthermore, the heterotrophic plate count in the control (group 1) showed no significant change in bacterial plate count, whereas there was a significant decrease in the gut bacterial plate count of the treated rat groups (2, 3, 4, and 5) after acclimatisation and after 7 days treatment, which was dependent on the duration.However, after further 14 days of treatment, there was a considerable increase in bacterial plate count in both groups. Furthermore, utilising the Clinical Laboratory Standard Institute (CLSI) standard to interpret the zone diameter of inhibition revealed a higher degree of multidrug resistance to the antibiotics used in this investigation, with Bacillus and Enterobacter being the most resistant bacteria. Importantly, molecular identification proved to be more precise and specific than convectional identification.

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