

**GUT MICROBIOME OF DIARRHOEIC UNDER-FIVE CHILDREN IN LAGOS
AND OGUN STATES, NIGERIA**

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13PCQ00507

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AND OGUN STATES, NIGERIA**

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**A THESIS SUBMITTED TO THE SCHOOL OF POSTGRADUATE STUDIES IN
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DEPARTMENT OF BIOLOGICAL SCIENCES, COLLEGE OF SCIENCE AND
TECHNOLOGY, COVENANT UNIVERSITY, OTA.**

MAY, 2021

ACCEPTANCE

This is to attest that this thesis is accepted in partial fulfilment of the requirements for the award of the degree of Doctor of Philosophy (Ph.D) in Microbiology, in the Department of Biological Sciences, College of Science and Technology, Covenant University, Ota, Ogun State.

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DECLARATION

I, **UGBOKO, HARRIET UNUAGBON (13PCQ00507)**, declare that I carried out this research under the supervision of Prof. Obinna C. Nwinyi and Prof. Solomon U. Oranusi of the Department of Biological Sciences, College of Science and Technology, Covenant University, Ota, Nigeria. I attest that the thesis has not been presented either wholly or partially for the award of any degree elsewhere. All sources of data and scholarly information used in this thesis are duly acknowledged.

UGBOKO, HARRIET UNUAGBON

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Signature and Date

CERTIFICATION

We certify that this thesis titled “**THE GUT MICROBIOME OF DIARRHOEIC UNDER-FIVE CHILDREN IN LAGOS AND OGUN STATES, NIGERIA**” was carried out by **UGBOKO, HARRIET UNUAGBON (13PCQ00507)** in the Department of Biological Sciences, College of Science and Technology, Covenant University, Ota, Ogun State, Nigeria under the supervision of Prof. Obinna C. Nwinyi and Prof. Solomon U. Oranusi. We have examined and found this work acceptable as part of the requirements for the award of Doctor of Philosophy (Ph. D) degree in Microbiology.

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DEDICATION

This research is dedicated to Almighty God for divine inspiration. Also, to my very supportive husband, Lucky N. Ugboko (FCA), who stood by me consistently over the years.

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LIST OF ABBREVIATIONS

API:	Analytical Profile Index
ATCC:	American Type Culture Collection
AIDS:	Acquired Immunodeficiency Syndrome
Bp:	Base pairs
CDC:	Centre for Disease Control and prevention
DAS:	Deep Amplicon Sequencing
DNA:	Deoxyribonucleic Acid
dNTP:	Deoxynucleoside triphosphate
DsDNA:	Double-stranded DNA
DTC:	Difficult to Culture
EDTA:	Ethylene Diaminetetraacetic Acid
Gb:	Giga bases
GBD:	Global Burden of Disease
gDNA:	Genomic DNA
HIV:	Human Immunodeficiency Virus
HMP:	Human Microbiome Project
IMGM:	Institute for Molecular Genetics Martinsried
LGA:	Local Government Area
MSS:	Metagenome Shotgun Sequencing
NCDC:	Nigeria Centre for Disease Control
NGS:	Next Generation Sequencing
NMDS:	Non-metric Multidimensional Scaling
NTC:	No Template control
OR:	Odds Ratio
ORS:	Oral Rehydration Solution
ORT:	Oral Rehydration Therapy

OTU:	Operational Taxonomic Unit
PCR:	Polymerase Chain Reaction
PE:	Paired End
PF:	Passed Filter
QC:	Quality Control
RNA:	Ribonucleic Acid
SAV:	Sequence Analysis Viewer
SBS:	Sequencing by Synthesis
SDG:	Sustainable Development Goals
IBM-SPSS: Sciences	International Business Machines-Statistical Package for the Social Sciences
SPRI:	Solid Phase Reversible Immobilization
SsDNA:	Single-stranded DNA
TE:	Tris-EDTA
TS:	Target Specific
UNICEF:	United Nations Children's Fund
VBNC:	Viable but not culturable
WHO:	World Health Organization

ABSTRACT

Diarrhoea is a major cause of morbidity and mortality in young children. This study aimed to investigate the gut microbiome of under-five diarrhoeic children in Lagos and Ogun States. Hospital records were obtained from the four healthcare facilities used. **Ethical approval was obtained from two tertiary institutions.** A total of 180 stool samples were taken from under-five diarrhoeic and non-diarrhoeic children from two states in approximately a ratio of four to one (4:1). Samples were analysed using standard, cultural and biochemical techniques. Isolates were further identified using the API 20E test. An Antibiogram profile of the pure isolates was conducted using the disks diffusion technique. Amplicon-based metagenomic analyses were carried out on DNA isolates obtained from stool samples of diarrhoeic and healthy children. The species richness and species abundance were determined using the Microbial Genomics Module version 4.0 (Qiagen). Data obtained from the questionnaires, hospital records, and antibiogram were analysed using logistic regression, Chi-square and student t-tests. This study showed an overall prevalence rate of 11.1% for diarrhoea in Lagos and Ogun States. Mother's educational status (OR= 11.459, P= 0.0001), mother's employment status (OR= 2.082, P= 0.025) and family income (OR= 7.613, P=0.0001), were the factors significantly associated with diarrhoea. A total of 279 isolates obtained were predominantly members of the Enterobacteriaceae. Others include *Pseudomonas*, *Staphylococcus*, *Bacillus*, *Acinetobacter*, and *Alcaligenes*. **Most of the bacteria isolated from the diarrhoeic children were resistant to multiple class of antibiotics; Penicillins, Sulonamides, Tetracyclines, and Cefuroxime in a decreasing order.** The isolates were more resistant to Ampicillin (83.4%), Augmentin (79.6%), Trimethoprim-Sulphamethoxazole 70.5%), and Tetracycline (65.6%) and more sensitive to Nitrofurantoin (68.9%), Gentamycin 67.2%), Ofloxacin (65.9%), and Ciprofloxacin (61.8%). The phylogenetic diversity revealed six bacterial phyla which include Firmicutes (51.7%), Bacteroidetes (9.1%), Proteobacteria (27.1%), Actinobacteria (11.5%), Fusobacteria (0.5%), and Verrucomicrobia (0.1%). The major classes in the Firmicutes were Bacilli (71%) and Clostridia 24%. The classes under Proteobacteria were mainly Gammaproteobacteria (99.9%) while Alphaproteobacteria occurred (0.1%). The two classes, Actinobacteria (50%) and Coriobacteriia (50%), in the phylum Actinobacteria, were represented equally. The phyla Bacteroidetes, Fusobacteria and Verrucomicrobia were represented by a single class each which is: Bacteroidia (100%), Fusobacteriia (100%) and Verrucomicrobiae (100%), respectively. The predominant phyla detected among the diarrhoeic samples were Firmicutes (50.4%) and Proteobacteria (37.2%). There was a remarkable increase in the abundance of Proteobacteria in the diarrhoeic group (37.2%), compared to the healthy group (6.5%) at $p < 0.0001$. The total number of different species presented decreased in the diarrhoeic children (341, 31%) and increased in the non-diarrhoeic children (774, 69%). Also, the proportion of *Escherichia coli*, *Shigella*, *Staphylococcus* and *Klebsiella* were in abundance in the diarrhoeic group. In contrast, *Bifidobacterium*, *Faecalibacterium*, *Lactobacillus*, *Clostridium* (sensu stricto), and *Bacteroides* significantly decreased in the diarrhoeic group. This suggests that diarrhoeal **diseases** thrive in the dysbiotic gut, which is characterised by the depletion of beneficial microbes and increased pathogens in the gut.

Keywords: Bacteria, Children, Diarrhoea, Gut Microbiome, Metagenome