Phylogenetic Analysis and Genetic Diversity of Bacteria Strains isolated from oral cavity of Malnourished Children: Insights from Comparative Genomics

Abiodun Jacob Osatogbe
Kebbi State University of Science and Technology, Aliero, Kebbi State, Nigeria.
Microbiology Department
Corresponding author: abioduntiojo@gmail.com
ORCID: 0000000315413142

Prof. Ahmed Ali Farouq
Usmanu Danfodiyo University, Sokoto State, Nigeria.
HoD Microbiology Department
ahmedaslamfarouq@gmail.com

Associate Prof. Daniel. D Attah
Kebbi State University of Science and Technology, Aliero, Kebbi State, Nigeria.
Faculty of life science,
danielattah10@gmai.com

Prof. Sule Sahabi Manga
Kebbi State University of Science and Technology, Aliero, Kebbi State, Nigeria.
Microbiology department;
ssmanga2000@gmail.com

DOI: 10.56201/jbgr.v9.no2.2023.pg77.96

Abstract

Background

Molecular Characterization of Bacteria Isolated from the Oral Cavities of In-Patient Malnourished Children At Specialist Hospital, Sokoto, Nigeria. Research conducted at Sokoto State Specialist Hospital looked into oral bacteria in malnourished children. Malnutrition stems from nutrient inefficiency, and diet influences health significantly. A microbiological method of isolation involves aseptic oral swabs for examination. Samples are collected by spinning the swab across the mouth for 15–20 seconds. Self-sealing polythene bags transport specimen containers to the lab, ensuring accurate results. Isolation uses nutrient agar, sorbitol, and broth agars. Gram staining, biochemical testing detect nutritious microorganisms on the agar-spread plate. The slide is dried and examined under a microscope with an oil immersion objective lens. With molecular characterization of bacteria isolates from the oral cavity of the patients using PCR.

Results

Haemophilus influenzae, Staphylococcus aureus, Escherichia coli, Streptococcus pyogenes, Cronobacter condiment, Photorhabdus luminscenes, Klebsiella aeruginosa, Bacillus tequillensis, Yersinia molderath, and Bacillus megaterium from the patient's oral cavity. Twenty patients' mouths yielded 10 bacterial species. Among bacterial isolates, 25% are Cronobacter condimenti. In oral samples, Klebsiella aeroginosa, Haemophilus influenza, Staphylococcus aureus, Escherichia coli, and Streptococcus pyogenes were 9.1%, 12.5%, and 9.1%, respectively, while Bacillus tequillenses and Yersinia molderath were 4.5%. Klebsiella aeroginosa was found in 15% of patients, Photorhabdus luminscenes in 10%, Haemophilus influenza in 10%, Staphylococcus aureus, Escherichia coli, Streptococcus pyogenes, Bacillus megaterium, Cronobacter condimenti, and Bacillus tequillensis in 5%. We identified ten bacterial strains based on colony from the morphology, biochemical assays, 16S rDNA sequence analysis, and species-specific PCR with phylogenetic tree of six of the isolated bacteria. Agarose gel electrophoresis of all the isolated bacteria shows 16S RRNA gene amplicon of approximately 1450 bp presentencing a separation pattern of PCR amplified genomic DNA. The bacteria belongs to Haemophilus spp, Escherichia spp, Photorhabdus spp, Klebsiella spp, Bacillus spp and Yersinia spp with variation in species level. Their query cover and percentage similarity ranges from 97.9 to 99.9 respectively for all the isolated bacteria. Sequence obtained were submitted to the NCBI data bank and were assigned an identification tally (accession) numbers.

Conclusions

Malnutrition affects millions of poor children around the world. It causes short-term disease and death and can affect cognitive function, economic productivity, and reproduction. Food security, healthcare access, and proper oral hygiene and nutrition are needed to combat malnutrition.

Keywords: *Sequence Children, Oral cavity, Bacteria, Malnutrition, Isolate, Specialist Hospital and In-patient.*

INTRODUCTION

Childhood under nutrition encompasses a wide range of nutritional issues, including underweight, wasting, and stunted growth (Wagnew et al., 2018). Wasted malnutrition, defined by low weight for height, can be caused by a recent nutritional shortage, such as insufficient food intake, or by a persistent infection that causes weight loss, such as diarrhoea. Being underweight, defined as having an insufficient weight for one's age, is a comprehensive indicator of metabolic wasting and malnutrition, defined as having a low height for a person's chronological age. Weight-for-height -3Z scores according to the median WHO growth recommendations, a mid-upper arm circumference (MUAC) measurement of 115 mm, the presence of obvious severe wasting, and the presence of nutritional oedema are all diagnostic criteria for extreme acute malnutrition. Guesh et al. (2018) conducted research. Noncommunicable diseases (NCDs) associated with malnutrition, such as lack of nutrition and obesity, account for roughly half of the additional burden of

malnutrition. There are four primary markers of malnutrition: wasting, stunting, underweight, and a lack of specific micronutrients. Underweight, defined as a low weight-for-age ratio, is a comprehensive indicator for assessing both wasting and stunting. Wasted malnutrition, also known as low weight-for-height malnutrition, is a type of acute malnutrition caused by a recent nutritional shortage, such as inadequate calorie consumption, or a recent infection, such as diarrhoea, which results in decreased weight and stunted growth (low height-for-age). Several indicators, such as weight-for-height -3Z scores based on the median WHO growth standard, a mid-upper-arm circumference (MUAC) measurement of 115 mm, clear signs of severe wasting, and the presence of nutritional oedema, are used to diagnose severe acute malnutrition (Fassikaw et al., 2022). The ratio of being lean to height is the characteristic that characterises wasting. Sudden and extreme weight loss is a common symptom, with the disease having the potential to last for an extended period of time. This syndrome is frequently caused by a lack of healthy meals or by lengthy and recurrent sickness episodes. If left untreated, the incidence of child wasting increases the likelihood of death. Stunting is a condition that causes a person's stature to be shorter than their chronological age. These issues emerge as the consequence of inadequate feeding and/or care in the first few months of life. Chronic or recurring micronutrient deficits are common in those with poor socioeconomic status, pregnancy issues, recurrent diseases, and/or restricted access to adequate food resources.The oral microbiome, which is mostly made up of bacteria that have developed tolerance to human immune systems, has been seen to exert an effect on the microbe that hosts it for its own benefit, as evidenced by the formation of dental cavities. The mouth cavity provides an ideal environment for the growth of many bacteria that are usually found within it. It provides a source of water and vital nutrients while also maintaining a reasonable temperature, according to Sherwood et al. (2013). The oral microbiota, which is made up of resident bacteria, has an adhesive feature that allows it to adhere to the tissues of the mouth. This adhesion acts as a barrier against the mechanical cleansing activity that occurs within the mouth cavity, preventing bacteria from being transferred to the stomach. It is worth emphasising that the stomach environment, which is distinguished by the presence of acidic substances, is harmful to acid-sensitive bacteria, ultimately leading to their extinction (Wang et al., 2014).The widespread occurrence of antibiotic-resistant microorganisms is a major source of concern. In the case of malnourished children (Daniluk et al., 2006), the oral cavity has been shown to be a breeding ground for superinfecting bacteria, which are frequently related to the emergence of broad and opportunistic diseases (Gonçalves et al., 2007). Furthermore, the spread of these microorganisms' resistance genes within the oral microbiota population, as well as the use or misuse of antimicrobial agents in combination with insufficient oral hygiene practices, can all contribute to the establishment of these microorganisms within the oral cavity (Gaetti-Jardim et al., 2010).It has been found that pathogenic bacteria like Klebsiella pneumoniae, Enterobacter, Pseudomonas aeruginosa, E. coli, and Proteus mirabilis are common in the mouth and are the main cause of respiratory infectious diseases (Azusa, 2022). Oral streptococci, according to Bryskier (2002), are a ubiquitous group of bacteria that colonise the human oral cavity and perform a vital function in preventing the colonisation of other bacteria, such as staphylococci. Certain streptococci strains cling strongly to the buccal mucosa and gingiva but have no affinity for tooth surfaces. The gingival crevice area, which includes the area around the tooth-supporting structures, is home to a wide variety of

anaerobic microbes. According to Rogers (2008), spirochetes and bacteria commonly colonise the mouth throughout puberty. The inquiry focuses specifically on the bacteria found in the oral cavities of malnourished youngsters.According to Daniluk et al. (2006) and Goncalves et al. (2007), the mouth is a great place for bacteria that cause systemic and opportunistic diseases so they can spread. In their investigation, Paster et al. (2009) used the HOMIM (Human Oral Microbe Identification Microarray) framework. However, the 16S rRNA gene array used in this work has a larger scope because it attempts to identify the most prevalent oral bacterial species while ignoring any specific clinical condition. This method was first created to look at the variety of microbes in oral samples. It has since been used to look at microbiota from medical settings (like gastrointestinal or skin flora), environmental settings (like soil, sludge, and wastewaters), and food industry samples. Furthermore, it can be used as a cost-effective and more effective substitute for cloning and sequencing the ribosomal gene. The progress of molecular biology processes has contributed to a greater scientific understanding of oral ecology. Oral ecology mapping is getting more comprehensive, incorporating numerous components such as the tongue, teeth, gums, and salivary glands. These structural features serve as colonies for several microorganism species (Attar, 2016). Furthermore, the transmission of these microorganisms' resistance genes within the oral microbiota, combined with the use or misuse of antimicrobial agents in conjunction with insufficient oral hygiene practices, might encourage the establishment of these microbes in the oral cavity (Gaetti-Jardim et al., 2010). The current study looked into the risk factors and molecular properties of bacteria isolated from the oral cavities of malnourished children being treated at a specialty hospital in Sokoto.

METHODS

A total of 20 patients, 14 boys and 6 girls, with a mean age of 3.4 years, who are predominantly malnourished youngsters. The person undergoing the procedure was advised to wait for thirty minutes before eating in the morning, drinking, flossing, brushing their teeth, or using mouthwash before the swab. The Puritan PurFlock's swabs the ultra (25-3606-U) was rapidly duplicated. A sterile swab was used to swab the anterior portion of the patients' tongues. Swabs almost never bend. To collect samples, the swab was spun around the inside of the oral cavity for 15-20 seconds (Liabeya et al., 2019). Specimen containers are shipped in self-sealing bags made of polyethylene (Brekle and Hartley, 2014). Specimens are sent immediately to the lab to avoid tampering with the results. To avoid altering results, specimens are sent to the lab immediately. Local storage guidelines apply if specimens cannot be relocated quickly. Isolation used nutrient, sorbitol, and nutrient broth agars. Gram staining and biochemical assays were used to identify bacteria on an agar-spread plate (Cheeseborough, 2006). A colony was placed on a free-of-contamination slide with sterile water and dried to generate a bacterial culture smear. The back of the slide burned several times. Smears are removed after 60 seconds. The smear was stained with Gram's iodine for 60 seconds before being drained and stored in 95% ethanol until the stain, which was crystal violet, vanished for 10 to 15 seconds before being cleansed with water. For 30 seconds, counterstain with safranin. A light microscope with an oil-immersed objective lens (x100) was used to examine the slides shortly after they had previously been held in water and dried out

(Cheesbrough 2006). The following are examples: assays were performed: indole, motility, methyl red reaction, voges proskauer, urease, oxydase, citrate utilisation, coagulase, and TSI. The 16S rRNA gene sequence was amplified by PCR using universal bacterial primers 16S RRNA F' sequence AGAGTTTGATCCTGGCTCAG length 20, barcode S1151 and 16S RRNA R' TACGGCTACCTTGTTACGACTT length 22, barcode S1152. Purified PCR products were sequenced and analysed using Blast and search. The phylogenetic analysis was conducted for the all bacteria isolated from the oral cavity using the method of Ferris et al., 2003.

RESULTS

In the current study, species of different families were isolated including ten families of both gram positive and gram negative bacteria *Haemophilus influenzae, Staphylococcus aureus, Escherichia coli, Streptococcus pyogenes, Cronobacter condimenti, Photorhabdus luminscenes, Klebsiella aeroginosa, Bacillus tequillensis, Yersinia molderath,* and *Bacillus megaterium*, as shown in table 1 which agreed with (Alghamdi, 2022), in the mouths of malnourished children, researchers found *Haemophilus influenzae, Staphylococcus aureus, Escherichia coli, Streptococcus pyogenes, Cronobacter condimenti, Photorhabdus luminscenes, Klebsiella aeroginosa, Bacillus tequillensis, Yersinia molderath,* and *Bacillus megaterium. Cronobacter condimenti* in the mouth was 25%. Oral samples had 4.5% *Bacillus tequillenses, Yersinia molderath, and Bacillus megaterium. Klebsiella aeroginosa, Haemophilus influenza, Staphylococcus aureus, Escherichia coli,* and *Streptococcus pyogenes* had 9.1%, 12.5%, and 9.1%, respectively. Patients had 5% of *Bacillus megaterium*, 5% of *Cronobacter condimenti,* and 5% of *Bacillus tequillensis.* They also had 15% of *Klebsiella aeroginosa, Photorhabdus luminscenes, Haemophilus influenza, Staphylococcus aureus, Escherichia coli, and Streptococcus pyogene.*

TABLE : 1 BIOCCHEMICAL REACTION OF BACTERIA ISOLATED FROM ORAL CAVITY OF MALNOURISHED CHILDREN

S/N	Shape	Grxm	CIT	MR	VP	GLU	LAC	SUC	FRUC	H2S	GAS	URE CAT	BACTERIA
1	Rod	+	+	-	+	+	+	+	+	-	+	-	+	*Klebsiella aeroginosa*
2	Rod	-	+	+	+	+	+	+	+	-	+	-	+	*Bacillus tequilensis*
3	Rod	-	-	-	+	+	-	-	-	-	-	-	-	*Yersinia molderatti*
4	Rod	-	-	+	-	-	+	+	+	-	+	-	-	*Bacillus megaterium*
5	Rod	+	+	-	+	+	+	+	+	-	-	-	+	*Cronobacter condimenti*
6	Rod	+	+	-	+	+	+	+	+	-	-	-	+	*Cronobacter condimenti*
7	Rod	+	+	-	+	+	+	+	+	-	-	-	+	*Cronobacter condimenti*
8	Cocci	+	+	-	+	-	+	+	-	-	-	+	+	*Staphylococcus aureus*
9	Rod	-	+	-	+	+	-	-	-	-	-	-	+	*Photorhabdus lumiinscences*
10	Rod	-	+	-	+	+	-	-	-	-	-	-	+	*Photorhabdus lumiinscences*
11	Rod	+	-	+	-	+	+	+	+	+	-	-	-	*Streptococcus pyogenes*
12	Rod	-	+	-	+	+	+	+	+	-	-	-	+	*Escherichia coli*
13	Rod	-	-	-	+	+	+	+	+	-	-	-	-	*Escherichia coli*
14	Rod	-	+	-	+	+	+	+	+	-	+	-	+	*Cronobacter condimenti*
15	Rod	-	+	-	+	+	-	-	-	-	-	-	+	*Photorhabdus lumiinscences*
16	Rod	-	-	-	+	+	+	+	-	-	-	-	+	*Haemophilus influenza*
17	Rod	-	+	-	+	+	+	+	+	-	+	-	+	*Cronobacter condimenti*
18	Rod	-	+	-	+	+	+	+	+	-	+	-	+	*Cronobacter condimenti*
19	Cocci	-	-	-	+	+	+	+	-	-	-	-	+	*Haemophilus influenza*
20	Bacilli	+	+	-	+	+	+	+	+	-	+	-	+	*Klebsiella aeroginosa*
21	Rod	+	-	+	-	+	+	+	+	+	-	-	-	*Streptococcus pyogenes*
22	Rod	-	-	-	+	+	+	+	-	-	-	-	+	*Haemophilus influenza*
23	Rod	-	-	+	-	+	+	+	+	-	-	-	+	*Escherichia coli*

24	Cocci	+	+	-	+	-	+	+	-	-	-	+	+	*Staphylococcus aureus*

NOTE: G.rxn: Gram reaction, CIT: Citrate; MR: Methyl red; VP:Voges praeuker; GLU: Glucose; LAC: Lactose; SUC: Sucrose; FRUC: Fructose; H_2S: Hydrogen Sulphide; GAS: Gas; UREA: Urease; CAT: Catalase.

TABLE: 2 Frequency of Isolates from the patients

Bacterial	No of Isolates	%	No of patients	%
Haemophilus influenza	3	12.5	2	10
Staphylococcus aureus	2	8.3	2	10
Escherichia coli	3	12.5	2	10
Streptococcus pyogenes	2	8.3	2	10
Cronobacter condiment	6	25	3	15
Photorhabdus luminscenes	3	12.5	2	10
Klebsiella aeroginosa	2	8.3	3	15
Bacillus tequillensis	1	4.5	1	5
Yersinia molderath	1	4.5	1	5
Bacillus megaterium	1	4.5	2	10

A total of 24 isolates were subjected to susceptibility tests, as follows: *Haemophilus influenza* (3 isolates), *Staphylococcus aureus* (2 isolates), *Escherichia coli* (3 isolates), *Streptococcus pyogenes* (2 isolates), *Cronobacter condiment* (6 isolates), *Photorhabdus luminscenes* (3 isolates), *Klebsiella aeroginosa* (2 isolates) while *Bacillus tequillensis*, *Yersinia molderath*, and *Bacillus megaterium* (all have 1 isolates each) as shown in the table above.

Band view of 16S rRNA gene sequene in gel with a binding dye

DNA fragments of the same length form a "band" on the gel, which can be seen by eye if the gel is stained with a DNA-binding dye. For example, a PCR reaction producing a 400400400 base pair (bp) fragment would look like this on a gel below Fig 1:

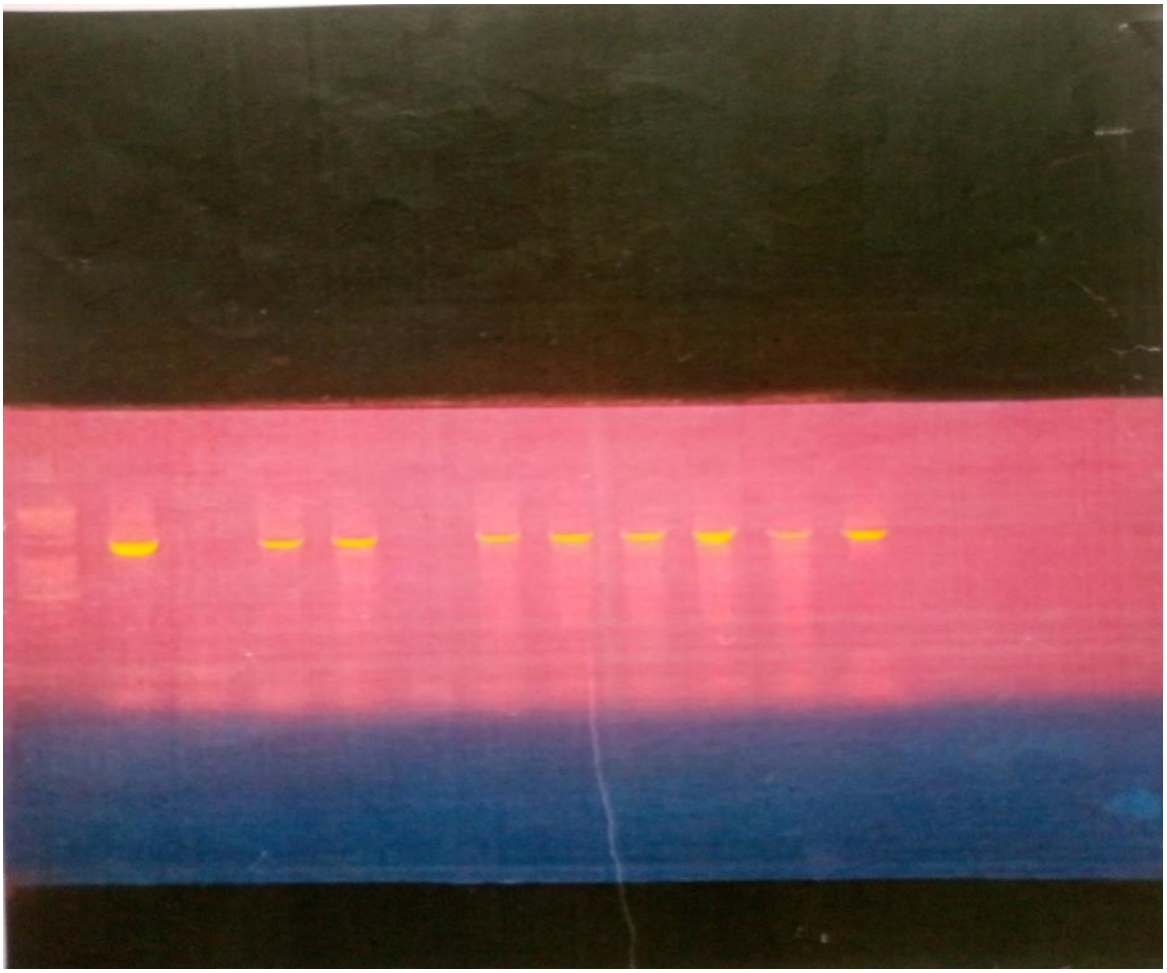


Fig: 1

Molecular Identification of 16SrRNA analysis

Agarose gel electrophoresis of all the isolated bacteria shows 16S RRNA gene amplicon of approximately 1450 bp presentinting a separation pattern of PCR amplified genomic DNA as shown above. The bacteria belongs to *Haemophilus spp, Escherichia spp, Photorhabdus spp, Klebsiella spp, Bacillus spp* and *Yersinia spp* with variation in species level. Their query cover and percentage similarity ranges from 97.9 to 99.9 respectively for all the isolatd bacteria as shown in the table below. Sequence obtained were submitted to the NCBI data bank and were assigned an identification tally (accession) numbers.

Sequence

This study presents a comprehensive phylogenetic analysis of Bacteria strains based on genetic sequences and identity percentages. The research explores the genetic diversity among different

bacteria isolates from oral cavity of malnourished children as seen in the figures below, with a focus on those obtained from diverse geographical regions. The analysis includes recently sequenced strains from this study and publicly available genomic data. The study reveals remarkable genetic similarity among bacteria strains and provides insights into their evolutionary relationships. The findings shed light on the global distribution and genetic conservation of this bacteria.

The table below appears to be a list of bacteria strains along with their respective accession numbers, E-values, identity percentages, and the country of origin. This data is typically used in constructing phylogenetic trees to understand the evolutionary relationships between these bacterial species.

Bacteria Species: The list includes different strains or isolates of bacteria, a bacterial species. Each strain has been identified by its accession number.

E-value: The E-value is a measure of the statistical significance of the alignment between the sequences. A lower E-value indicates a more significant match. In your data, E-values are quite low (0.0 or very close to 0), suggesting very strong sequence matches between these strains.

Identity (%): The identity percentage represents the similarity between the sequences. In this context, it's showing the genetic similarity between the bacteria strains. The higher the percentage, the more similar they are at the genetic level.

Country of Origin: This column indicates the geographical origin or source of each bacteria strain.

Fig 2: Blast comparison between *Haemophilus* spp gene identified and other *Haemophilus* spp in Gene Bank using NCBI blast

S/N	*Haemophilus* spp	**Accession no**	**E-value**	**Identity (%)**	**Country of origin**
1	*Haemophilus influenzae*	LT686763.1	0.00	99.9	
2	*Haemophilus influenzae*	MW261312.1	0.0	98.8	
3	*Haemophilus influenzae*	-	0.0	98.1	This study
4	*Haemophilus influenzae*	KC332179.1	0.0	97.9	
5	*Haemophilus influenzae*	LN589737.1	0.0	97.9	

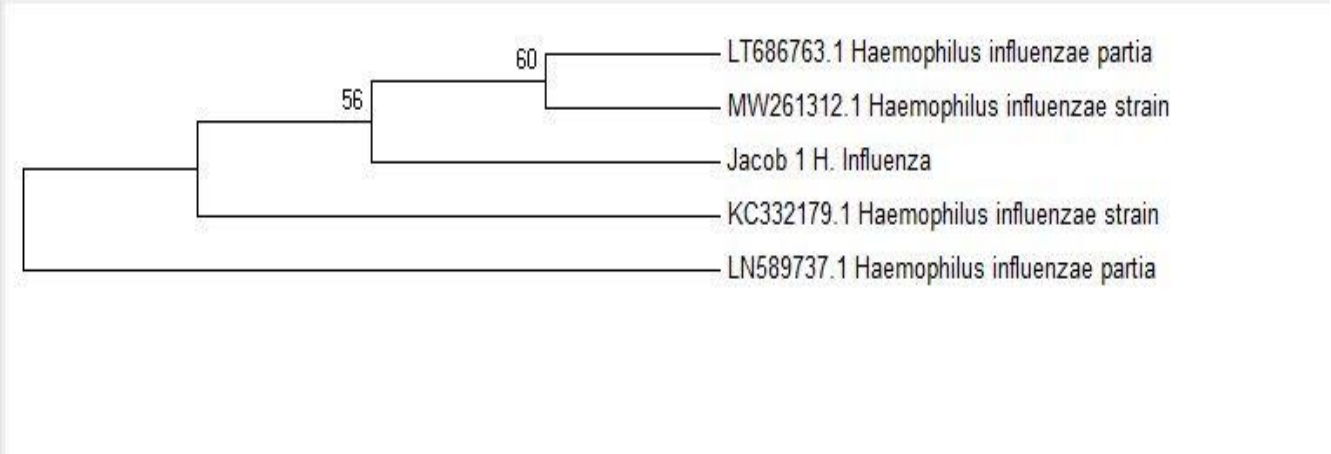


Fig 3: Blast comparison between *Escherichia* spp gene identified and other *Escherichia* spp in Gene Bank using NCBI blast

S/N	*Escherichia spp*	**Accession no**	**E-value**	**Identity (%)**	**Country of origin**
1	*Escherichia coli*	MH197074.1	0.00	99.9	
2	*Escherichia coli*	MG566070.1	0.0	98.8	
3	*Escherichia coli*	MG566068.1	0.0	98.1	
4	*Escherichia coli*	-	0.0	97.9	This study
5	*Escherichia coli*	MT320434.1	0.0	97.9	

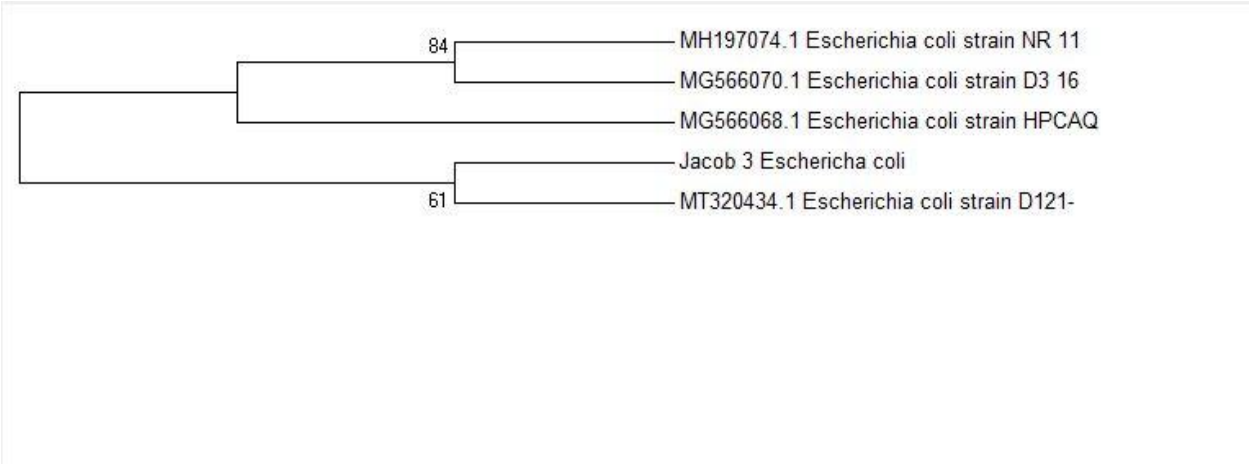


Fig 4: Blast comparison between *Photorhabdus* spp gene identified and other *Photorhabdus* spp in Gene Bank using NCBI blast

S/N	*Photorhabdus spp*	**Accession no**	**E-value**	**Identity (%)**	**Country of origin**
1	*Photorhabdus luminescens*	KP224434.1	0.00	98.9	
2	*Photorhabdus luminescens*	KP224439.1	0.0	98.8	
3	*Photorhabdus luminescens*	KP224432.1	0.0	98.8	
4	*Photorhabdus luminescens*	KP224435.1	0.0	98.7	
5	*Photorhabdus luminescens*	-	0.0	97.9	This study

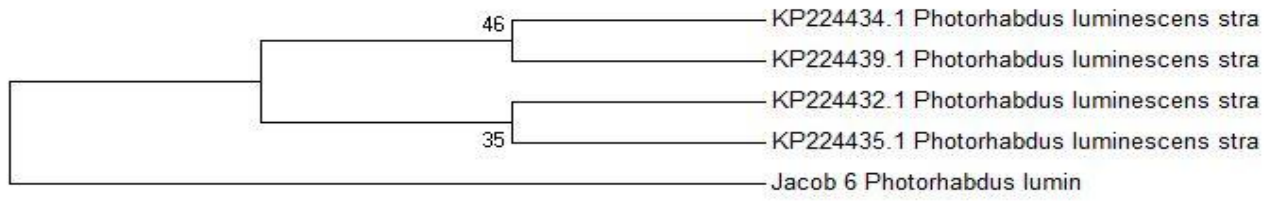


Fig 5: Blast comparison between *Klebsiella* spp gene identified and other *Klebsiella* spp in Gene Bank using NCBI blast

S/N	***Klebsiella spp***	**Accession no**	**E-value**	**Identity (%)**	**Country of origin**
1	*Klebsiella aeriginosa*	-	0.00	99.9	This study
2	*Klebsiella pneumoniae*	MH569438.1	0.0	98.8	
3	*Klebsiella pneumoniae*	CP052252,1	0.0	98.1	
4	*Klebsiella pneumoniae*	HQ670758.1	0.0	97.9	
5	*Klebsiella pneumoniae*	MG818749.1	0.0	97.9	

Fig 6: Blast comparison between *Bacillus* spp gene identified and other *Bacillus* spp in Gene Bank using NCBI blast

S/N	***Bacillus spp***	**Accession no**	**E-value**	**Identity (%)**	**Country of origin**
1	*Bacillus tequilensis*	-	0.00	99.9	This study
2	*Bacillus tequilensis*	MK392042.1	0.0	98.8	
3	*Bacillus tequilensis*	KF135456.1	0.0	98.1	
4	*Bacillus tequilensis*	MN598645.1	0.0	97.9	
5	*Bacillus tequilensis*	LS998695.1	0.0	97.9	

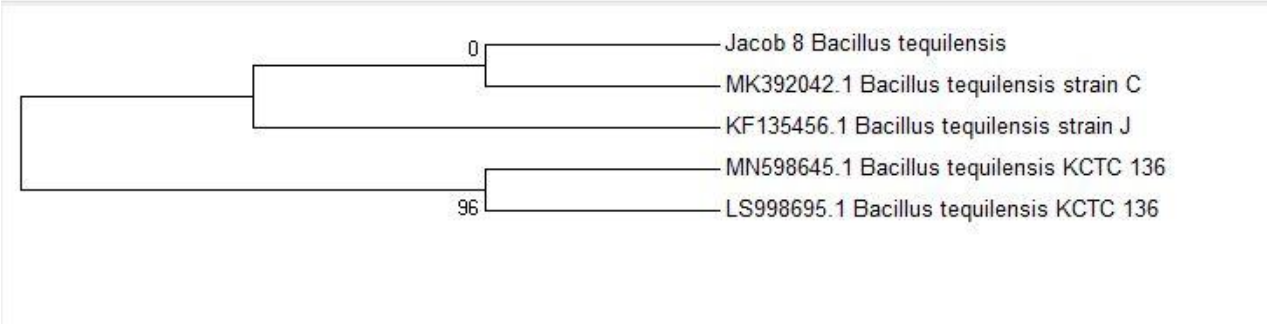
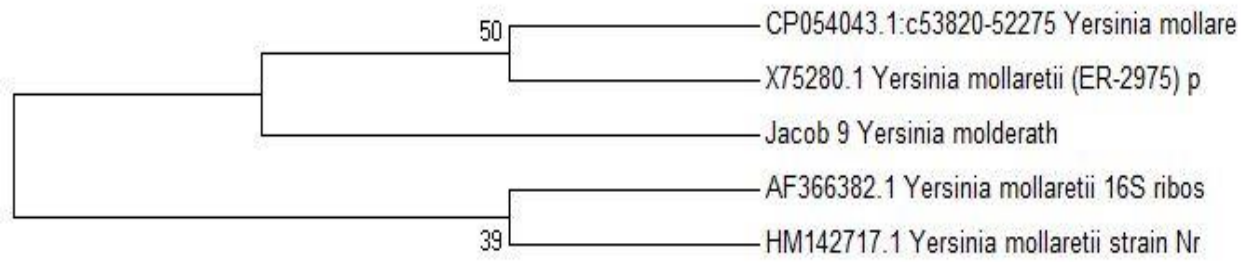


Fig 7: Blast comparison between *Yersinia* spp gene identified and other *Yersinia* spp in Gene Bank using NCBI blast

S/N	***Yersinia spp***	**Accession no**	**E-value**	**Identity (%)**	**Country of origin**
1	*Yersinia molleratti*	CP054043.1	0.00	99.1	
2	*Yersinia molleratti*	X75280.1	0.0	98.8	
3	*Yersinia molleratti*	-	0.0	98.1	This study
4	*Yersinia molleratti*	AF366382.1	0.0	97.9	
5	*Yersinia molleratti*	HM142717.7	0.0	97.9	



Phylogenetic analysis of 16S RNA gene sequene

The evolutionary relationship is indicated in the phylogenetic analysis as seen in FIG 1-6 above. Further phylogenetic features in the bacteria showed a closed relationship in cluster of all bacteria isolated in this study. The relationship noticed was that between all the isolated bacteria with Jacob and all other gene spp are in the same clade.

DISCUSSION

The human oral cavity can be thought of as a microcosm, with diverse ecological niches such as the anterior and posterior surfaces on the tongue, the mucosal epidermal layer of the firm mouth, the palate's soft part, and supra-gingival debris on tooth surfaces. These niches are occupied by a varied variety of microbes, including fungi, viruses, and bacteria (Alghamdi, 2022). According to Chen et al. (2010), 1100 different taxa were found and catalogued in the Man Oral Microbiome Database. The buccal cavity is home to a varied microbial population dominated by Firmicutes, bacterial genera, Proteobacteria, Spirochaetes, and Fusobacteria, despite a limited representation of other phyla (Bik et al., 2010). Figure 2 to 6 depicts this composition. Certain bacteria in this category provide significant benefits, while others have the potential to cause severe infections. Certain bacteria have the ability to go from a beneficial to a harmful lifestyle, resulting in severe oral cavity infections (Ahn et al., 2012). These bacteria have developed an intimate

relationship with human body over the period of time and represents the single most abundant microflora in human microbiome structure (Rahman et al., 2015). As a result, these microbes exhibit opportunistic traits.

CONCLUSIONS

The results of this investigation confirmed that the oral cavity of malnourished patients, and particularly in-patients could harbor microorganisms, and these bacteria are frequently implicated in multiresistant, systemic, oral or nosocomial infections. Consequently, in this study, we were able to isolate and identify several oral bacterial strains which belonged to the species.

Lists of Abbreviations

WHO- World Health Organisation

FTT- Failure To Thrive

FAO –Food and Agriculture Organization

UNICEF – United Nation Children Emergency Fund

WFP – World food Programme

IFAD – International Food and Agriculture Development

FMOH – Federal Ministry of Health

LMIC – Low and Middle Income Countries

SAM – Severe and Acute Malnutrtion

NCD – Neglected and Communicable Diseases

SMoH – State Ministry of Health

PEM - Protein-Energy Malnutrition

MUAC – Mid and Upper Arm Circumference

DECLARATION

I, **Abiodun Jacob Osatogbe,** declare that this work is the result of my research, effort and the best of my knowledge it has not been presented by any other person for the award of any degree except where due acknowledgements have been made.

References

Ahn, J., Chen, C.Y., Hayes, R.B., 2012. Oral microbiome and oral and gastrointestinal cancer risk. Cancer Causes Control. 23 (3), 399–404

Ahmed, T., Haque, R., Shamsir Ahmed, A. M., Petri, W. A. Jr., and Cravioto, A. (2009). Use of metagenomics to understand the genetic basis of malnutrition. *Nutr. Rev.* 67, 201–206.

Alghamdi, S. (2022). Isolation and identification of the oral bacteria and their characterization for bacteriocin production in the oral cavity. In Saudi Journal of Biological Sciences (Vol. 29, Issue 1, pp. 318–323). https://doi.org/10.1016/j.sjbs.2021.08.096

Azusa Haruta, Miki Kawada-Matsuo, Mi Nguyen-Tra Le, Mineka Yoshikawa, Toshiki Kajihara, Koji Yahara, Norikazu Kitamura (2022) Disinfectant Susceptibility of Third-Generation-Cephalosporin/Carbapenem-Resistant Gram-Negative Bacteria Isolated from the Oral Cavity of Residents of Long-Term-Care Facilities, Bacteriology Research Article, 14 December 2022 https://doi.org/10.1128/aem.01712-22

Beisel WR, (1996). Nutrition and immune function: overview. J Nutr 126: 2611S–2615S

Berkman DS, Lescano AG, Gilman RH, Lopez SL, Black MM. (2002). Effects of stunting, diarrhoeal disease, and parasitic infection during infancy on cognition in late childhood: a follow-up study. Lancet;359:564–57.

Bertrand M (2009). "DUHL Olga Anna (dir.), Amour, sexualité et médecine aux XVe et XVIe siècles, Dijon, Editions Universitaires de Dijon, 2009". *Genre, Sexualité & Société (n°2). doi:10.4000/gss.1001. ISSN 2104-3736.*

British Society for Antimicrobial Chemoterapy. BSAC methods for antimicrobial Susceptibility testing. [online]. Version 7.1. London: BSAC; 2008. Available from: <http://www.bsac.org.uk/_db/_documents/version_7_1_february_2008.pdf>

Bryskier A. (2002) Viridans group streptococci: a reservoir of resistant bacteria in oral cavities Clinical Microbiology and Infection Volume 8, Issue 2, February, Pages 65-69

Chen T., Yu W.H., Izard J., Baranova O.V., Lakshmanan A., Dewhirst F.E., 2010. The human oral microbiome database: a web accessible resource for investigating oral microbe taxonomic and genomic information Database (Oxford). 6, 13

CLSI (2018) CLSI Guideline M02 – Performance Standards for Antimicrobial Disk Susceptibility Tests (13th edn). Wayne, PA: CLSI

Cui L, Morris A, Ghedin E (2013). *"The human mycobiome in health and disease". Genome Medicine.* ***5*** *(7): 63. doi:10.1186/gm467. PMC 3978422. PMID 23899327.*

Daniluk T, Fiedoruk K, Zciepuk M, Zaremba ML, Rozkiewicz D, Cylwik-Rokicka D, et al. Aerobic bacteria in the oral cavity of patients with removable dentures. Adv Med Sci. 2006;51(Suppl. 1):86-90

Ellen Cristina Gaetti-Jardim; Antônio Carlos Marqueti; Leonardo Perez Faverani; Elerson Gaetti-Jardim Júnior, (2010) Antimicrobial resistance of aerobes and facultative anaerobes isolated from the oral cavity, J. Appl. Oral Sci. 18 (6) • Dec 2010 • https://doi.org/10.1590/S1678-77572010000600004

Fassikaw Kebede, Tsehay Kebede, Belete Negese, Atitegeb Abera1,Getahun Fentaw, Ayalew Kasaw (2022) Incidence and predictors of severe acute malnutrition mortality in children aged 6–59 months admitted at Pawe general hospital,Northwest Ethiopia. PLoS ONE17(2): e0263236. https://doi.org/10.1371/journal.pone.0263236

Federal Ministry of health. FMOH. National Strategic Health Development Plan 2018 –2022. Ensuring healthy lives and promoting the wellbeing of Nigerian populace at all ages. https://www.nipc.gov.ng/ViewerJS/#../wp-content/uploads/2019/02/NSHDP-II-final-version-health-plan.pdf [Accessed 30 Jun 2021]

Federal Ministry of Health, Nigeria. Health Facilities Registries. FMOH (2021)., Abuja. Available from http://hfr.health.gov.ng/facilities/hospitals [Accessed 13th March 2021]

Felsenstein J. (1985). Confidence limits on phylogenies: An approach using the bootstrap. Evolution 39:783-791.

Feris K, Ramsey P, Frazar C, Moore JN, Gannon JE, Holben WE (2003) Differences in hyporheic zone microbial community structure along a heavy-metal contamination gradient. Appl Environ Microbiol J 69(9):5563–5573. https://doi.org/10.1128/AEM.69.9.5563-5573.2003

Food and Agriculture Organization of the United Nations, World Food Programme and International Fund for Agricultural Development. State food insecurity world. FAO WFP. IFAD. 2012;2012.

Gonçalves MO, Coutinho-Filho WP, Pimenta FP, Pereira GA, Pereira JAA, Mattos-Guaraldi AL, et al. Periodontal disease as reservoir for multi-resistant and hydrolytic enterobacterial species. Lett Appl Microbiol. 2007;44:488-94

Guesh G., Degu G., Abay M., Beyene B., Brhane E. (2018): Survival status and predictors of mortality among children with severe acute malnutrition admitted to general hospitals of Tigray, North Ethiopia: aretrospective cohort study BMC Res Notes; 11: 832. https://doi.org/10.1186/s13104-018-3937-x PMID: 30477540

Guideline WHO (2013). Updates on the management of severe acute malnutrition in infants and children. Geneva (Switzerland): WHO; 2013.

Howell E, Waidmann T, Birdsall N, Holla N, Jiang K. (2020). The impact of civil conflict on infant and child malnutrition, Nigeria, 2013. Matern Child Nutr Jul;16(3): e12968–e12968.

Judd L. Walson and James A. Berkley (2018). *The impact of malnutrition on childhood infections* 0951-7375 Copyright The Author(s). Published by Wolters Kluwer Health, Inc. Volume 31;3

Liesbeth Vogel, Gwendolytt Jories, Shirley Tviep, Alex Koek and Letiie Dijkshoorn (1999), RAPD typing of Klebsiella pneumoniae, Klebsiella oxytoca, Serratia marcescens and Pseudomonas aeruginosa isolates using standardized reagents, Department of Medical Microbiology, Leiden University Medical Center, Leiden, The Netherlands, Clin Micvobiol Infect; 5: 370-276

Malek M, Sharifiyan M (1990).. Malnutrition and failure to thrive. Iran J Pediatr. 3:203–231.

Noble JM, Scarmeas N, Papapanou PN (2013). "Poor oral health as a chronic, potentially modifiable dementia risk factor: review of the literature". *Current Neurology and Neuroscience Reports.* ***13*** *(10): 384. doi:10.1007/s11910-013-0384-x. PMC 6526728. PMID 23963608.*

"Oral health: A window to your overall health". *Mayo Clinic. Retrieved 2019-04-16.*

Pietropaoli D, Del Pinto R, Ferri C, Ortu E, Monaco A (2019). "Definition of hypertension-associated oral pathogens in NHANES". *Journal of Periodontology.* ***90*** *(8): 866–876. doi:10.1002/JPER.19-0046. PMID 31090063. S2CID 155089995.*

Rahman, M., Islam, M. N., Islam, M. N., & Hossain, M. S. (2015). Isolation and identification of oral bacteria and characterization for bacteriocin production and antimicrobial sensitivity. *Dhaka University Journal of Pharmaceutical Sciences*, *14*(1), 103–109. https://doi.org/10.3329/dujps.v14i1.23742

Reshad A. Failure to thrive. Med Ther. 1991;8:42–47

Rodríguez L, Cervantes E, Ortiz R. (2011). Malnutrition, and gastrointestinal and respiratory infections in children: a public health problem. Int J Environ Res Public Health. ;8(4):1174–205.

Rogers AH (2008). Molecular Oral Microbiology. *Norfolk, UK: Caister Academic Press. ISBN 9781904455240. OCLC 170922278.*

Rogers AH, ed. (2008). Molecular Oral Microbiology. *Caister Academic Press. ISBN 978-1-904455-24-0.*

Sherwood L, Willey J, Woolverton C (2013). *Prescott's Microbiology (9th ed.). New York: McGraw Hill. pp. 713–721. ISBN 9780073402406. OCLC 886600661*.

Stephenson LS, Latham MC, Ottesen EA (2000). Global malnutrition. Parasitology; 121 Suppl: S5- 22.

Spoelstra MN, Mari A, Mendel M, Senga E, Van Rheenen P, Van Dijk TH, Reijngoud DJ, Zegers RGT, Heikens GT, Bandsma RHJ (2012) Kwashiorkor and marasmus are both associated with impaired glucose clearance related to pancreatic b-cell dysfunction. Metabolism;61:1224–30.

Turnbaugh, P. J., Ridaura, V. K., Faith, J. J., Rey, F. E., Knight, R., and Gordon, J. I. (2009). *The effect of diet on the human gut microbiome: a metagenomics analysis in humanized gnotobiotic mice*. *Sci. Transl. Med.* 1, 6ra14.

UNICEF. UNICEF data: Malnutrition. 2020 Available at https:// www.unicef.org/nutrition/index_sam.html. Accessed April 01, 2020.

United Nations Children's Fund United (2013). Improving Child Nutrition: The achievable imperative for global progress. UNICEF: 4

van der Kam S, Salse-Ubach N, Roll S, Swarthout T, Gayton-Toyoshima S, Jiya NM, (2016) Effect of Short-Term Supplementation with Ready-to-Use Therapeutic Food or Micronutrients for Children after Illness for Prevention of Malnutrition: A Randomised Controlled Trial in Nigeria. PLoS Med.;13(2):e1001952..

Wagnew F., Tesgera D., Mekonnen M., Abajobir A (2018). A. Predictors of mortality among under-five children with severe acute malnutrition, Northwest Ethiopia: an institution based retrospective cohort study. Archives of Public Health; 76: 64., https://doi.org/10.1186/s13690-018-0309-x PMID: 30275951.

Wagnew F, Debrework T, Mengistu M, Abajobir AA (2018). Predictors of mortality among under-five children with severe acute malnutrition, northwest Ethiopia: an institution based retrospective cohort study. Arch Public Health;76(1):64.

Watson JL, Berkley JA. (2018) The impact of Malnutrition on childhood infections. Curr Opin Infect Dis.; 31(3): 231–236.

World Health Organization. (2021). Malnutrition Key fact sheet. WHO 2018 Available from: https://www.who.int/news-room/fact-sheets/detail/malnutrition [Accessed 18th February 2021]

World Health Organization. WHO, (2020) child growth standards and the identification of severe acute malnutrition in infants and children. 2009. ISBN: 978 92 4 159816 3. Available at https://www.who. Int/nutrition/publications/severe malnutrition/9789241598163/en/. Accessed March 30, 2020.

World Health Organization(2021).. Malnutrition Key fact sheet. WHO 1st April 2020. Available from: https://www.who.int/news-room/fact-sheets/detail/malnutrition [Accessed 16th January 2021]

World Health Organization (WHO, 2021); Updates on the management of severe acute malnutrition in infants and children, accessed, September 2021. https://www.who.int/publications-detail- redirect.

LETTER OF CONSENT FOR RESEARCH

Metagenomic Studies of Bacterial Isolates from Oral Cavity of Malnourished Children among In-Patient of Specialist Hospital Sokoto.

Oshatogbe Abiodun Jacob, 07036259071, and abioduntiojo@gmail.com

Dr. Aliero A.A , 08080337133 and adamualieroa@gmail.com

Dear All,

You are invited to participate in a research study, the purpose of this research is to;

1. Isolate bacteria from the oral cavity of patients.
2. Determine drug susceptibility/resistance of the bacterial isolates.
3. Determine bacterial profiles in the oral cavity of the patients.
4. Determine the titre of IgG and IgE as a measure of the status of their immunity.

The Kebbi State University of Science and Technology and review Board approved the study and its procedures. The study involves no foreseeable risks or harm to you.

Your participation in this study is Voluntary. You are under no obligation to participate. You may withdraw at any time. By returning the completed surveys implies consent for participating in the study. To maintain anonymity, please do not write your name on any of the materials.

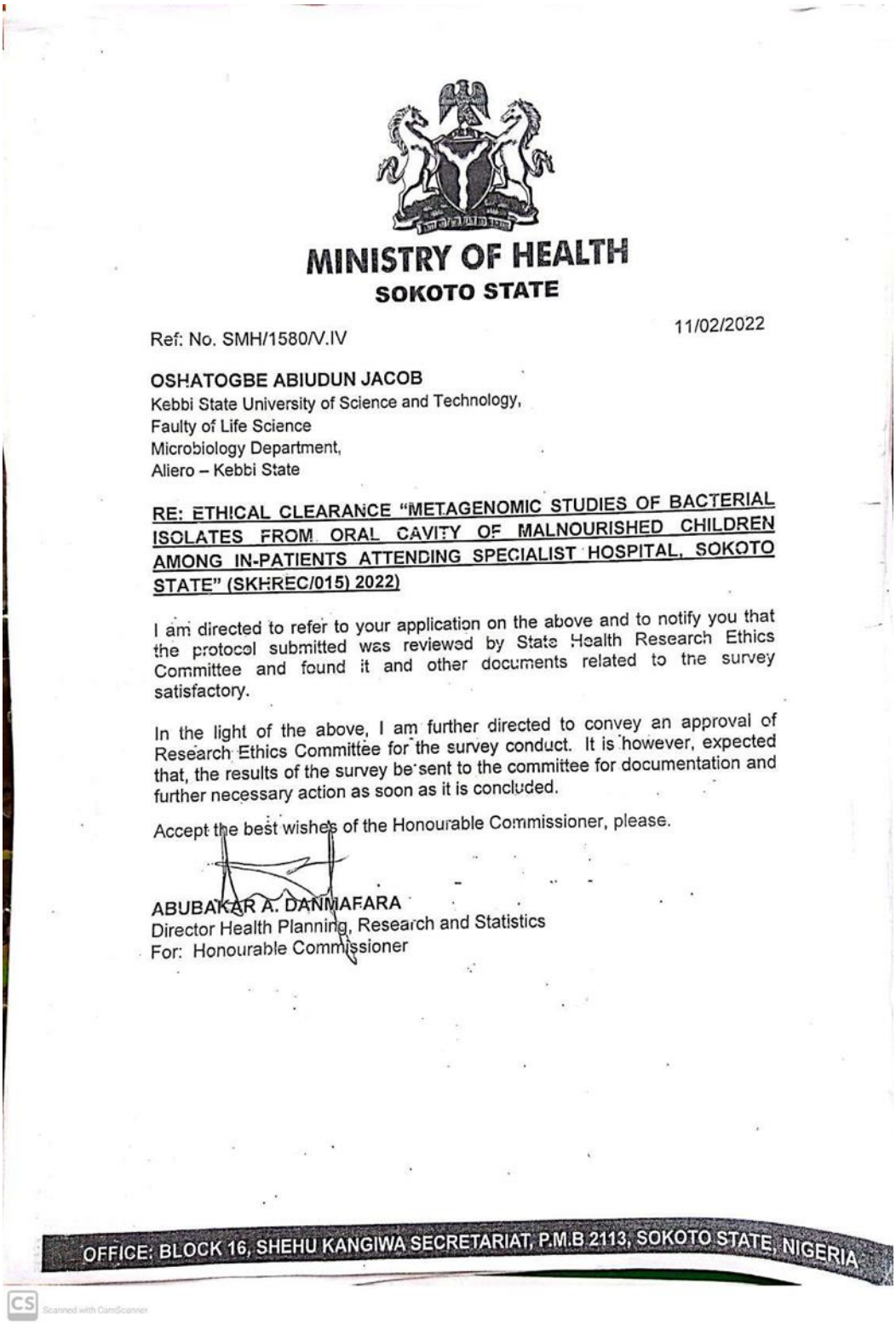
The completed study will be reported in the aggregate. Confidentiality will be maintained. All data will be collected by **Reasearch's Name** stored in a secure place and will be destroyed in three years.

I have read this informed letter and voluntarily consent to participate in this study.

If your participaton in our survey has caused you to feel uncomfortable in any way, or if our survey prompted you to consider personal matters about which you are concerned, we encourage you to take advantage of the confidential counseling services offered at Morrouah University. You can contact a counselor at 732-571-

Yours Sincerely, _____

Name,_____
Address:_____
Phone Number_____



MINISTRY OF HEALTH
SOKOTO STATE

Ref: No. SMH/1580/V.IV

11/02/2022

OSHATOGBE ABIUDUN JACOB
Kebbi State University of Science and Technology,
Faulty of Life Science
Microbiology Department,
Aliero – Kebbi State

RE: ETHICAL CLEARANCE "METAGENOMIC STUDIES OF BACTERIAL ISOLATES FROM ORAL CAVITY OF MALNOURISHED CHILDREN AMONG IN-PATIENTS ATTENDING SPECIALIST HOSPITAL, SOKOTO STATE" (SKHREC/015) 2022)

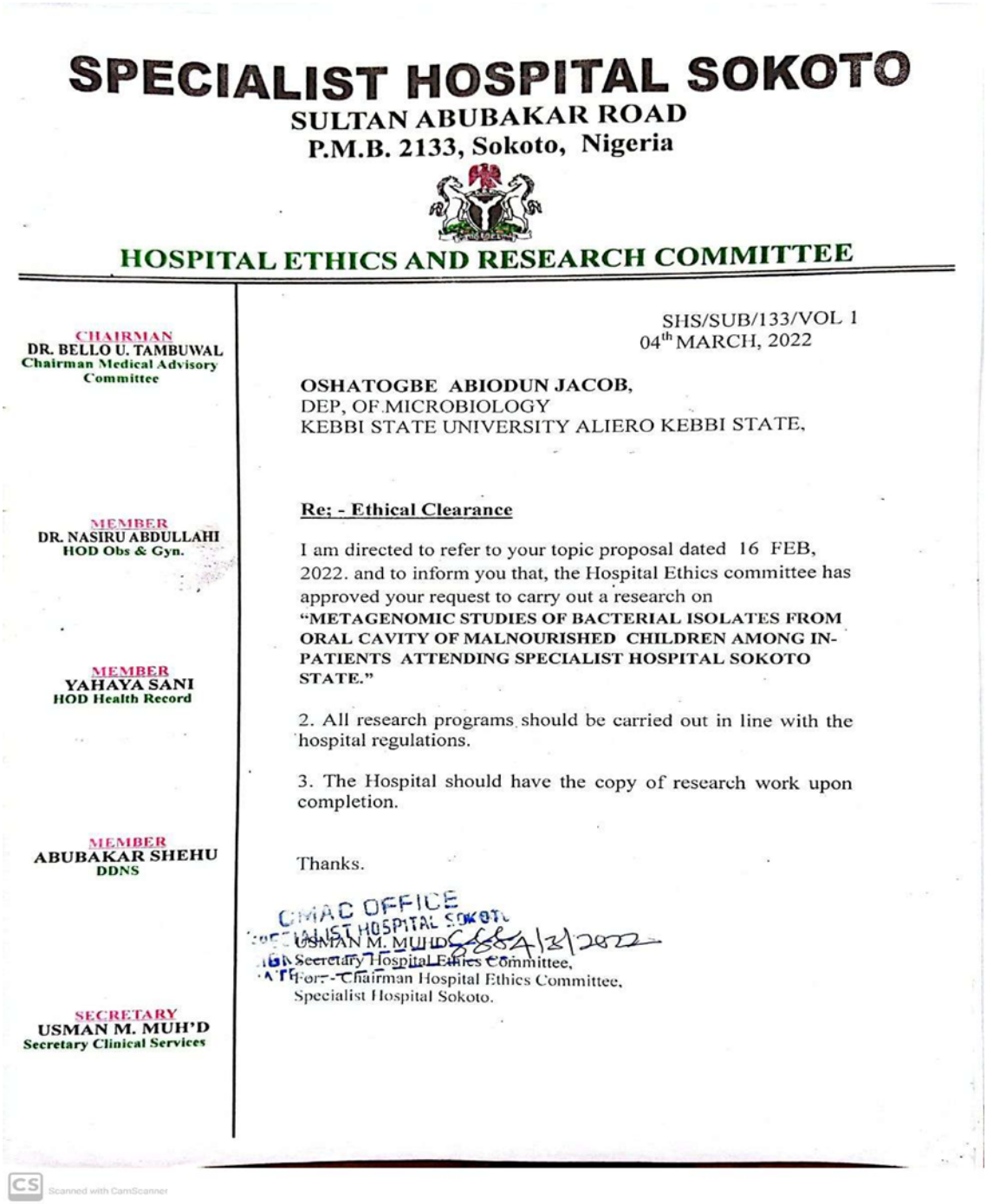
I am directed to refer to your application on the above and to notify you that the protocol submitted was reviewed by State Health Research Ethics Committee and found it and other documents related to the survey satisfactory.

In the light of the above, I am further directed to convey an approval of Research Ethics Committee for the survey conduct. It is however, expected that, the results of the survey be sent to the committee for documentation and further necessary action as soon as it is concluded.

Accept the best wishes of the Honourable Commissioner, please.

ABUBAKAR A. DANMAFARA
Director Health Planning, Research and Statistics
For: Honourable Commissioner

OFFICE: BLOCK 16, SHEHU KANGIWA SECRETARIAT, P.M.B 2113, SOKOTO STATE, NIGERIA



SPECIALIST HOSPITAL SOKOTO

SULTAN ABUBAKAR ROAD
P.M.B. 2133, Sokoto, Nigeria

HOSPITAL ETHICS AND RESEARCH COMMITTEE

CHAIRMAN
DR. BELLO U. TAMBUWAL
Chairman Medical Advisory Committee

MEMBER
DR. NASIRU ABDULLAHI
HOD Obs & Gyn.

MEMBER
YAHAYA SANI
HOD Health Record

MEMBER
ABUBAKAR SHEHU
DDNS

SECRETARY
USMAN M. MUH'D
Secretary Clinical Services

SHS/SUB/133/VOL 1
04th MARCH, 2022

OSHATOGBE ABIODUN JACOB,
DEP, OF MICROBIOLOGY
KEBBI STATE UNIVERSITY ALIERO KEBBI STATE.

Re; - Ethical Clearance

I am directed to refer to your topic proposal dated 16 FEB, 2022. and to inform you that, the Hospital Ethics committee has approved your request to carry out a research on **"METAGENOMIC STUDIES OF BACTERIAL ISOLATES FROM ORAL CAVITY OF MALNOURISHED CHILDREN AMONG IN-PATIENTS ATTENDING SPECIALIST HOSPITAL SOKOTO STATE."**

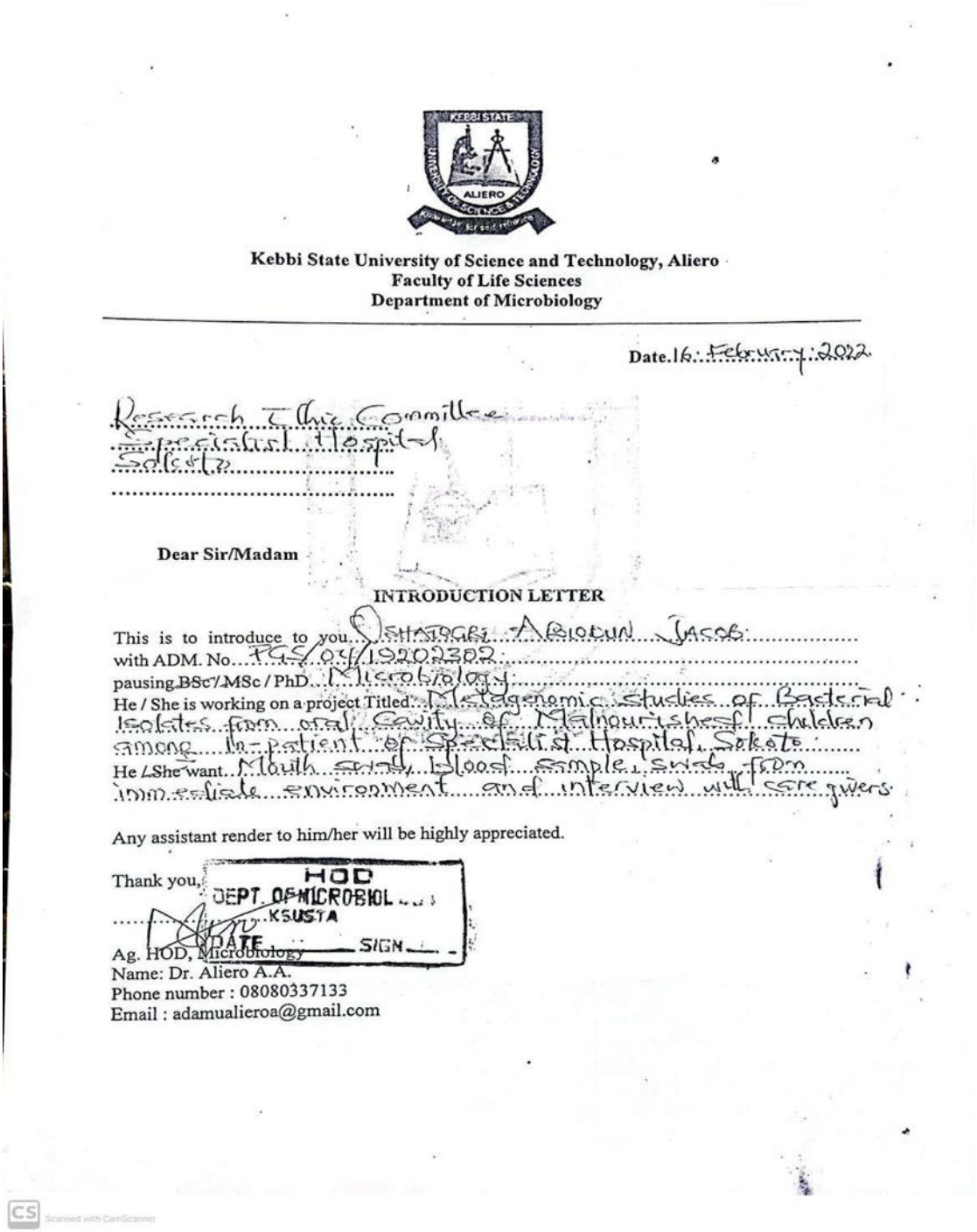
2. All research programs should be carried out in line with the hospital regulations.

3. The Hospital should have the copy of research work upon completion.

Thanks.

CMAC OFFICE
SPECIALIST HOSPITAL SOKOTO

USMAN M. MUH'D 24/3/2022
Secretary Hospital Ethics Committee,
For: Chairman Hospital Ethics Committee,
Specialist Hospital Sokoto.



Kebbi State University of Science and Technology, Aliero
Faculty of Life Sciences
Department of Microbiology

Date. 16th February, 2022.

Research Ethics Committee
Specialist Hospital
Sokoto

Dear Sir/Madam

INTRODUCTION LETTER

This is to introduce to you Oshatogbi Abiodun Jacob
with ADM. No. KGS/04/19202302
pursuing BSc/MSc / PhD Microbiology
He / She is working on a project Titled Metagenomic studies of Bacterial Isolates from oral Cavity of Malnourished children among In-patient of Specialist Hospital, Sokoto
He /She want Mouth swab, blood sample, swab from immediate environment and interview with care givers

Any assistant render to him/her will be highly appreciated.

Thank you,

HOD
DEPT OF MICROBIOL
KSUSTA
DATE SIGN

Ag. HOD, Microbiology
Name: Dr. Aliero A.A.
Phone number : 08080337133
Email : adamualieroa@gmail.com

Scanned with CamScanner

✓ ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Ethical clearance was obtained from the Ethical Review Committee of Sokoto State Ministry of Health, Kebbi State University of Science and Technology Aliero, And Sokoto State Specialist Hospital Sokoto. The objective and purpose of the study were explained to officials at the Kebbi

Satate University of Science and Technology Aliero, Sokoto Ministry of Health, Specialist Hospital Sokoto and written permission consent was obtained from the study participants. For those study participants whose age is below 18 years consent to participate in the study was obtained from their parent and care givers during the samples collection time as seen below.

- ✓ **AVAILABILITY OF DATA AND MATERIAL**

These can be made available on request.

- ✓ **COMPETTING INTEREST**

No competing interest

- ✓ **FUNDING**

No fund was received/Not applicable

- ✓ **AUTHOR CONTRIBUTION**

Abiodun Jacob Osatogbe- Kebbi State University of Science and Technology, Aliero, Kebbi State.

- **Manuscript Idea Conception and Design**

Associate Prof. Daniel. D Attah - Kebbi State University of Science and Technology, Aliero, Kebbi State.

- **Manuscript Draft and Supervision**

Prof. Sule Sahabi Manga- Kebbi State University of Science and Technology, Aliero, Kebbi State.

- **Manuscript Draft and Supervision**

Prof. Ahmed Ali Farouq- Usmanu Danfodiyo University, Sokoto State**.**

- **Manuscript Design and Supervision**

✓ **ACKNOLEDGEMENT**

I want to express my gratitude to Prof. A. A. Farouq for giving me the chance to work in the field of human microbial metagenomics. Prof. A. A. Farouq has always given me a great deal of latitude to create my own projects and has provided me with unwavering support as I conduct my research. Every time I encountered a setback, his upbeat and creative attitude has been a source of inspiration. Over the past years, he has been a motivating mentor. My co-supervisors Dr. S. S. Manga, Dr. D. D. Attah, Dr. Obaro, and HOD Microbiology Dr. A. Aliero held deserve my gratitude for their time, helpful criticism, and suggestions on my work. Additionally, I want to thank the entire Microbiology Department staff and my departmental peers for the inspiring atmosphere, especially Mr. Joseph, Hajiya Zara, and Mrs. Martina for the fruitful brainstorming sessions. We appreciate the efforts of the Laboratory staff, especially Mal Dabai Ahmed for his prompt assistance when required. Thank you to the department's entire administrative team for providing prompt assistance when required. Additionally, I want to thank the functional genomics facility (CAMRET) for making it simple for me to access top-notch sequencing technologies and for supporting my research.

✓ **AUTHORS INFORMATION**

Abiodun Jacob Osatogbe

Microbiology Department

Kebbi State University of Science and Technology, Aliero, Kebbi State.

+2348077730479

abioduntiojo@gmail.com

Associate Prof. Daniel. D Attah

Faculty of life science,

Kebbi State University of Science and Technology, Aliero, Kebbi State.

+2348038350460

danielattah10@gmai.com

Prof. Sule Sahabi Manga

Microbiology department;

Kebbi State University of Science and Technology, Aliero, Kebbi State.

+2347064853240

ssmanga2000@gmail.com

Prof. Ahmed Ali Farouq
HoD Microbiology Department

Usmanu Danfodiyo University, Sokoto State.

+2348161653400

ahmedaslamfarouq@gmail.com