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Original Article

Metagenomic Analysis of Bacterial Communities in Water and Soil of the Fulani and non-Fulani in Nigeria

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Abstract

Introduction: Interactions between environmental factors (water and soil) and humans are inevitable, particularly in rural and semi-urbanized regions. As such, knowledge on the microbial constituents of these environmental factors is key to understanding potential risk to public health. However, the microbial profile of soil and water present in vulnerable human communities in Nigeria is currently unknown. This study sought to investigate the composition of soil and water microbiota in the environment inhabited by recently studied human communities (the Fulani nomadic group and the urbanized Jarawa ethnic group) and estimate the contribution of these environmental factors to the microbiome of the aforementioned human communities.

Methodology: Soil and water samples were collected from the Fulani and non-Fulani community in Jengre (Plateau State, Nigeria) and Jos (Plateau State, Nigeria), respectively. Genomic DNA was extracted from these environmental samples, followed by Illumina sequencing of the V4 region of the 16S rRNA gene and bioinformatics analysis via Quantitative Insights into Microbial Ecology QIIME.

Results: There is abundance of Proteobacteria (43%) signature members in soil samples obtained from both human communities. Analysis of the water samples revealed the abundance of Proteobacteria, particularly in water sourced from the borehole (Fulani). *Pseudomonas* (30%) had higher relative abundance in the drinking water of the Fulani.

Conclusions: The drinking water of the Fulani could be a potential health risk to the studied Fulani community. Factors that increase the abundance of public health threats and health risk, such as hygiene practices, soil and water quality need to be studied further for the improvement of health in vulnerable populations.

Key words: Soil; water; environmental microbiota; Fulani; public health; Nigeria.

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Introduction

Africa has the fastest growing need for water of any continent in the world [1], with a rising tide towards urbanization on a yearly basis. Despite this impressive feat, ultimately, resources will be needed to have been developed and maintained to cater for the daily needs of the rising population, one of which is very easy to take for granted by the developed world: water. In Africa, water is not just an important part of life, water is life [2]. This assertion comes against the backdrop of death prevalence in Africa linked to water-borne diseases due to the poor quality of water derived from unimproved water sources [1]. It is believed that if the water quality problem can be solved, there will be a concomitant reduction in diseases common in Africa including cholera, typhoid, diarrhea and tuberculosis [3].

The soil plays a major role in water quality. Hygiene levels and anthropogenic activities on the soil could result in the development of soil erosion that bear fruit as run-off to water bodies used by unsuspecting humans for their domestic activities [4], thereby contributing to increased risk of water-borne and water-associated diseases particularly in vulnerable populations. Therefore, in order to achieve a core aspect of the sustainable development goals (SDGs) of the UN by 2030 (that is, to ensure availability and sustainable management of water and sanitation for all [5,6], efforts are needed to create an environment that supports better hygiene, water quality and sanitation. A study that provides information on the microbial profile, and hence, microbial quality of water and soil of the studied groups is needed.

In a previous study [7], we showed that the lifestyle of a nomadic pastoral group (the Fulani) contributes immensely to the composition and predicted function of their gut microbiota. Furthermore, since nomadic pastoralism is the major source of livelihood in the Fulani community, followed by agricultural activities (mainly farming), it suffices to assume that there is a

constant interaction between the soil and the Fulani community. The water used for drinking and domestic activities are sourced from a nearby stream and a borehole recently built by a non-governmental organization. In comparison, the non-Fulani community (Jarawa) including students and workers within the major hospital of an urban region in Nigeria. Individuals within this community rely on treated water from the hospital and water sourced from the well for their domestic activities.

In this study, we aim to investigate the microbial profile of soil and water used by the Fulani and non-Fulani for consumption and domestic activities. Knowledge about the structure of the soil and water microbiota is needed to identify hotspots of public health threats in the environment of the vulnerable Fulani community.

Methodology

Study area

The Fulani are inhabitants of a remote area (Pabaman-shanu) in Jengre (10.2950° N, 8.7784° E), a town about 60 kilometres from Jos (9.8965° N, 8.8583° E) situated in the North-central region of Nigeria. On the other hand, the Jarawa (an ethnic group) were residents of Jos University Teaching Hospital and its proximate environment.

Sample Collection

Two soil samples were obtained from two sites each within the environment occupied by the Fulani; close to the households, and the grazing site. Two soil samples were also obtained at two separate points in the environment occupied by the non-Fulani: the farmland, and the land close to the residence of the non-Fulani. A water sample was collected from the well commonly used by the non-Fulani. The well is located between the farmland and the houses, and it has an attached lid to cover the well. In the environment occupied by the Fulani, two water samples were collected from the borehole installed with a hand pump (the Fulani community's source of drinking water) and stream (used for other domestic activities). The samples were stored at – 80°C until further processing.

DNA Extraction, 16S rRNA Gene Amplification and Sequencing

Total genomic DNA isolation from environmental samples was achieved via enzymatic and mechanical lysis, followed by DNA extraction with the use of the MagnaPure LC DNA III Isolation kit (Bacteria, Fungi; Roche, Mannheim, Germany) according to previously described protocols [8,9] on a MagNA Pure LC 2.0 Instrument (Roche Diagnostics, Mannheim, Germany). Briefly, the environmental samples were mixed with a Bacterial Lysis buffer and bead-beating in a Magna Lyser instrument for mechanical lysis. Following incubation with lysozyme and Proteinase K for enzyme lysis, samples were heat inactivated for 10 minutes. Total DNA was eluted in 100 µL catridge plates and stored for further analysis. To prepare DNA libraries, the V4 hypervariable region of the 16S rRNA gene was targeted during PCR amplification, using the primer set 515F-5′ -GTGCCAGCMGCCGCGGTAA and 806R-5′ -GGACTACHVGGGTWTCTAAT (Eurofins, Germany). This was followed by the introduction of barcode sequences for each sample by using the normalized PCR product as the template for indexing PCR. Afterwards, purification of the pooled sequencing library, quantification of the pooled sequencing library using the Quantus Fluorometer (Promega, Mannheim, Germany), and quality check on an Agilent 2100 Bioanalyzer (Agilent Technologies, Waldbronn, Germany) was achieved with the use of a 7500 DNA chip. Sequencing wad done on the Illumina MiSeq platform (Illumina, Eindhoven, Netherlands) according to previously described protocols [8].

Analysis of Soil and Water Microbiota

Raw, paired-end sequence reads were processed with the Quantitative Insights into Microbial Ecology (QIIME) pipeline (v1.9.1) on the Galaxy server of the Medical University of Graz (https://galaxy.medunigraz.at/) using standard settings. Concisely, paired-end reads were merged for further processing, followed by sequence filtering to remove reads of low quality (Phred score < 25). Adapters were removed with Cutadapt (v1.16) [10], while chimeric sequences were removed with USEARCH v6.1 [11]. Clustering steps into Operational Taxonomic Units (OTUs) was achieved with the use of the QIIME open reference pipeline at 97% sequence similarity, while the UCLUST algorithm was used for the taxonomy assignment. After singleton removal, further downstream data analyses for alpha and beta diversity as well as statistical calculations were derived from the core diversity analysis workflow of QIIME (core_diversity.py) and from Calypso (http://cgenome.net) [12] which uses the R package vegan [13] for the implementation of alpha diversity metrices. Alpha diversity was computed by using Chao1 index, Shannon and Simpson's Index of diversity. Rarefaction was achieved with a minimal sequencing depth of 13100 sequences per sample. Beta

diversity was determined by computing Bray-Curtis distances of OTU-level relative abundances for Principal Co-ordinate Analysis (PCoA) and Redundancy Analysis (RDA). P-values were calculated by using the non-parametric pairwise t-test which in turn uses 999 Monte Carlo simulations, and were corrected for multiple testing with the use of False Discovery Rate (FDR) and/or Bonferroni method.

Results

Soil Microbiome in Fulani and non-Fulani Environment

Quality filtering of the sequence data from the soil samples yielded 100,590 high-quality sequence reads with a mean of 25148 ± 6187 reads/soil sample. The reads were binned into 5332 OTUs. No significant difference in the soil microbial alpha diversity (p = 0.674) was observed when the soils from the Fulani and non-Fulani surroundings were compared. Beta diversity analysis using PCoA and RDA plots of Bray-Curtis distances showed no significant distinction between the soil samples.

Analysis of Fulani soil samples revealed the preponderance of signatures of Proteobacteria (M = 55.79%, SD = 8.25), and Bacteroidetes (M = 6.01%, SD = 3.6) while non-Fulani soil analysis showed the dominance of Firmicutes signatures (M = 14.76%, SD = 1.85) and Germmatimonadetes (M = 16.27%, SD = 10.38) (Figure 1). At the genus level, many of the genera belonging to Proteobacteria were greater in proportion in the soil samples from the Fulani environment than the soil samples obtained from the non-Fulani environment [*Pseudomonas* (Fulani 4.7%, non-Fulani 0.1%), *Devosia* (Fulani 6%, non-Fulani 0.1%), *Comamonadaceae*_other (Fulani 3.1%, non-Fulani 0.3%), *Mesorhizobium* (Fulani 4.2%, non-Fulani 0%)] (Figure 2).

Figure 1. Microbial relative abundance of phylum-classified soil microbiota from soils obtained from the environment of the Fulani and non-Fulani.

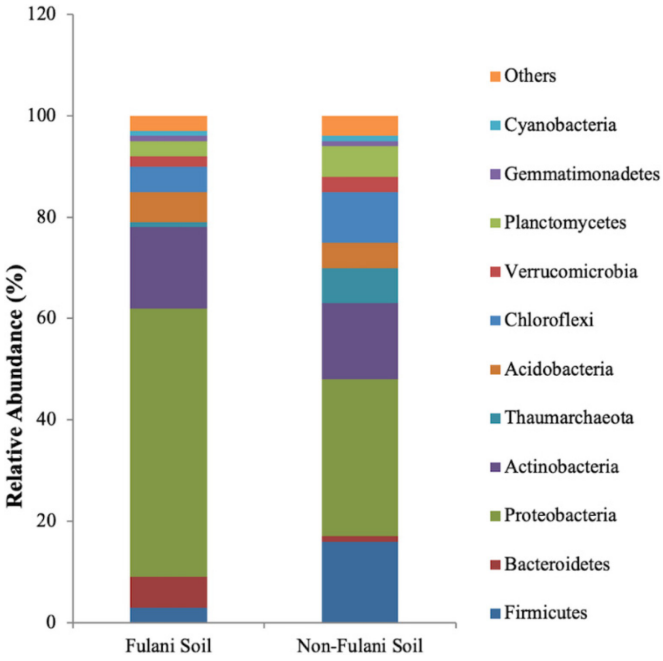
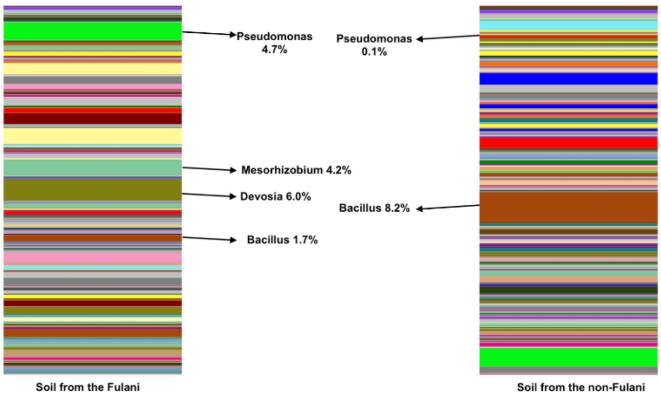


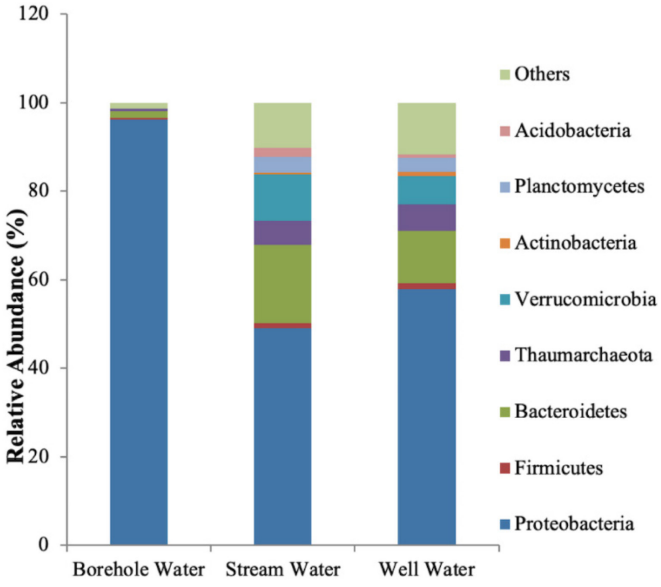
Figure 2. Relative abundance of *Pseudomonas*, *Devosia*, *Comamonadaceae*_other, and *Mesorhizobium* in the soil obtained from the environment of the Fulani and non-Fulani.



Water Microbiome in Fulani and non-Fulani Environment

Quality filtering of the sequence data from the water samples yielded 117,057 high-quality reads with a per sample read mean of 39,019 ± 4572. The reads were binned into 2562 OTUs based on 97% similarity.

Figure 3. Microbial relative abundance of phylum-classified water microbiota from Fulani (borehole and stream) and on-Fulani (well) water sources.



Water from the borehole in the Fulani environment was dominated by Proteobacteria signatures (94%) (Figure 3). The most dominant members of the Proteobacteria observed include *Pseudomonas* (30.25%), *Vogesella* (24.08%), *Pseudogulbenkiania* (21.83%), *Legionella* (4.12%), and *Azospirillum* (1.4%) (Figure 4).Water from the stream was dominated by signatures of Proteobacteria (47.9%) and Bacteroidetes (15.5%), Verrucomicrobia (10.4%), and Thaumarchaeota (3.4%) The water from the well (from the non-Fulani) was dominated by signatures of Proteobacteria (59.3%), Bacteroidetes (10.9%), and Verrucomicrobia (6.2%) (Figure 4).

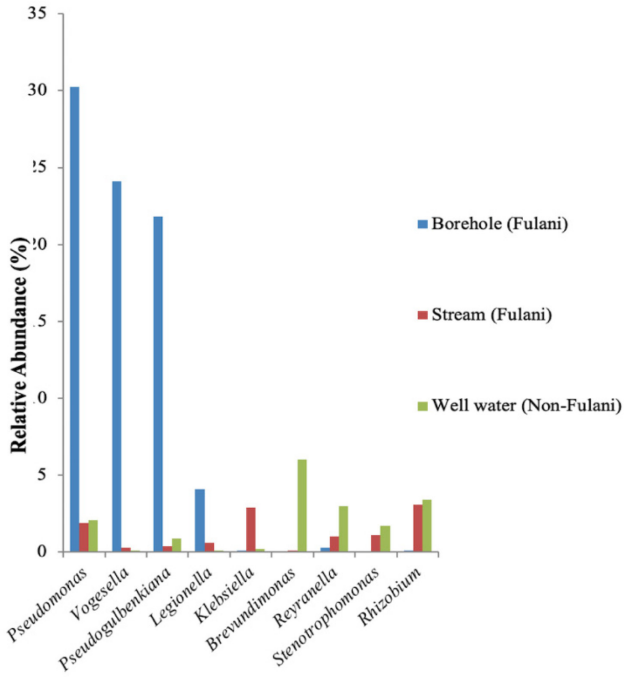
Although members of the Proteobacteria were the most dominant in the three water sources, signatures of *Pseudomonas* were not as dominant in the water from the well (2.06%; non-Fulani) and stream (1.91%; Fulani), as the water from the borehole (30.25%; Fulani) (Figure 4).

No significant difference in the microbial community diversity was observed in the water samples sourced from Fulani and non- Fulani environment.

Discussion

There has been a recent trend towards the maintenance of soil health as one of the ways of achieving a favorable climate [14]. However, anthropogenic activities not only affect soil health, but soil quality, which in turn could affect water quality that ultimately affect the lives of vulnerable populations. Similar to the soil microbiome, the water microbiome is increasingly being studied for climate change reasons and also for public health surveillance [15]. This is crucial, because the health of a community is intricately linked with drinking water and water used for other domestic activities. Here, we present findings from the analysis of the soil and water microbiota present in the environment of a previously studied vulnerable population [7].

Figure 4. Microbial relative abundance of genus-classified microbiota in water samples



Members of the phylum Proteobacteria were more abundant in the soils obtained from the Fulani. It is generally believed that there is an association between the evolution of the soil and human microbiome over time, and that close contact with the soil results in the sharing and replenishment of the different microbiomes with microbes, genes, and molecules, a surprising attribute that is still developing [16]. Due to this sentiment, it is tempting to suggest that there is an association between the Fulani habit of consuming unwashed fruits laden with soil components and the abundance of Proteobacteria in the gut of the Fulani. Although we did not test the aforementioned assumption, one cannot deny the fact that the unhygienic practices of the nomadic Fulani (which include random consumption of unwashed foods and fruits containing soil components) would contribute to their vulnerability.

The microbial community of stream water in this study was diverse, mirroring the report of previous studies on the stream water microbiome [17,18], with Proteobacteria dominating. Members of the phylum Proteobacteria were particularly abundant in the water sourced from the borehole that serves as one of the drinking water sources in the Fulani environment. The high abundance of Proteobacteria have been observed in many other groundwaters globally [19,20].

One prominent member of the Proteobacteria that was of a high relative abundance was *Pseudomonas* (30%) in water obtained from the borehole. The borehole is the major source of drinking water in the Fulani community. Many of the members of Proteobacteria are well known water-borne pathogens and causative agents for diseases of public health concern [21]. For instance, *Pseudomonas*—a notorious biofilm former—was one of the pathogens detected in water distribution systems and is associated with opportunistic infections [21]. It is likely that *Pseudomonas,* which is small in size (about 0.5 μM by 1.5 μM) compared to other Gram-negative pathogens, escaped the filtering system of the borehole and formed biofilms along the borehole pipes with occasional

shedding as the water moved up the pipe during the manual (hand pump) suction.

Our study has some limitations. First, sequence data were generated from the Illumina-based sequencing of the V4 region of the 16S rRNA gene. However, we were unable to resolve bacterial taxonomy to the species and strain level. Recently, taxonomic resolution to the species and strain level have been reported to be accurately derived from long read sequencing of the whole length of the 16S rRNA gene [22]. We hope that in subsequent studies, we will be able to utilize this technology as prices and error rates reduce. Secondly, as the sample size of the soil and water samples collected during our study was relatively small, we agree that a subsequent large-scale study on the soil and water of the unique environments studied will be needed to validate our findings. Nevertheless, our study shows for the first time the potential impact of soil and water quality, as well as the indirect impact of hygiene behavior on health risk of human communities in Nigeria. Information derived from this study, although small-scale, might just be enough to kick-start measures to prevent outbreaks in vulnerable populations in Nigeria.

Conclusions

This study provides information on at least one of the risk factors that could contribute to the vulnerability of the Fulani, which is, the quality of soil and the quality of water used for drinking and domestic activities. This information is necessary to drive and trigger actionable policies aimed at protecting the lives of this vulnerable population in Nigeria.

References

1. UNDESA. International decade for Action "Water for life." (2014) Available: https://www.un.org/waterforlifedecade/africa.shtml. Accessed 6 October 2019.
2. Naraya D, Patel R, Schafft K, Rademacher A, Koch-Schulte S (2000) Can Anyone Hear Us? Oxford. Oxford University Press 354 p.
3. World Health Organization (2004) Water, sanitation and hygiene links to health. Facts and figures. Available: https://www.who.int/water_sanitation_health/publications/facts2004/en/. Accessed 10 October 2019.
4. Issaka S, Ashraf MA (2017) Impact of soil erosion and degradation on water quality: a review. Geol Ecol Landscapes. 1: 1–11.
5. Lautensach AK. (2011) United Nations Millennium Development Goals. In Newman J, editor. Green Ethics and Philosophy: An A-to-Z Guide. California. SAGE Publications, Inc.
6. United Nations (2015) Transforming our world: the 2030 Agenda for Sustainable Development. A Resolution adopted by the General Assembly on 25 September 2015. Available: https://undocs.org/A/RES/70/1. Accessed 6 October 2019.
7. Afolayan AO, Ayeni FA, Moissl-Eichinger C, Gorkiewicz G, Halwachs B, Högenauer C (2019) Impact of a Nomadic Pastoral Lifestyle on the Gut Microbiome in the Fulani Living in Nigeria. Front Microbiol. 10: 2138.
8. Klymiuk I, Bambach I, Patra V, Trajanoski S, Wolf P (2016) 16S Based Microbiome Analysis from Healthy Subjects' Skin Swabs Stored for Different Storage Periods Reveal Phylum to Genus Level Changes. Front Microbiol 7: 2012.
9. Kump P, Wurm P, Gröchenig HP, Wenzl H, Petritsch W, Halwachs B, Wagner M, Stadlbauer V, Eherer A, Hoffmann KM, Deutschmann A, Reicht G, Reiter L, Slawitsch P, Gorkiewicz G, Högenauer C (2018) The taxonomic composition of the donor intestinal microbiota is a major factor influencing the efficacy of faecal microbiota transplantation in therapy refractory ulcerative colitis. Aliment Pharmacol Ther. 47: 67–77.
10. Martin M (2011) Cutadapt removes adapter sequences from high-throughput sequencing reads. EMBnet.journal. 17:10.
11. Edgar RC, Haas BJ, Clemente JC, Quince C, Knight R (2011) UCHIME improves sensitivity and speed of chimera detection. Bioinformatics. 27: 2194–2200.
12. Zakrzewski M, Proietti C, Ellis JJ, Hasan S, Brion M-J, Berger B, Krause L (2017) Calypso: a user-friendly web-server for mining and visualizing microbiome-environment interactions. Bioinformatics. 33:782–783.
13. Oksanen J, Blanchet FG, Kindt R, Legendre P, Minchin PR, O'Hara RB, Simpson GL, Solymos P, Stevens MHH, Wagner H (2019) Vegan Community Ecology Package. R Packag version 2-5-5. Available https://cran.r-project.org/web/packages/vegan/vegan.pdf. Accessed 16 Jul 2019.
14. Dubey A, Malla MA, Khan F, Chowdhary K, Yadav S, Kumar A, Sharma S, Khare PK, Khan ML (2019). Soil microbiome: a key player for conservation of soil health under changing climate. Biodivers Conserv 28: 2405–2429.
15. Hull NM, Ling F, Pinto AJ, Albertsen M, Jang HG, Hong PY, Konstantinidis KT, LeChevallier M, Colwell RR, Liu WT (2019) Drinking Water Microbiome Project: Is it Time? Trends Microbiol 27: 670–677.

16. Blum WEH, Zechmeister-Boltenstern S, Keiblinger KM. (2019). Does Soil Contribute to the Human Gut Microbiome? Microorganisms 7: 287.
17. Medeiros JD, Cantão ME, Cesar DE, Nicolás MF, Diniz CG, Silva VL, Vasconcelos AT, Coelho CM (2016) Comparative metagenome of a stream impacted by the urbanization phenomenon. Braz J Microbiol 47: 835–845.
18. Ayeni FA, Biagi E, Rampelli S, Fiori J, Soverini M, Audu HJ, Cristino S, Caporali L, Schnorr SL, Carelli V, Brigidi P, Candela M, Turroni S (2018) Infant and Adult Gut Microbiome and Metabolome in Rural Bassa and Urban Settlers from Nigeria. Cell Reports. 23:3056–3067.
19. Sheng Y, Wang G, Zhao D, Hao C, Liu C, Cui L, Zhang G (2018) Groundwater Microbial Communities Along a Generalized Flowpath in Nomhon Area, Qaidam Basin, China. Groundwater. 56: 719–731.
20. Itävaara M, Salavirta H, Marjamaa K, Ruskeeniemi T (2016) Geomicrobiology and Metagenomics of Terrestrial Deep Subsurface Microbiomes. In: Advances in applied microbiology. 1–77 pp.
21. Falkinham JO, Hilborn ED, Arduino MJ, Pruden A, Edwards MA (2015) Epidemiology and ecology of opportunistic premise plumbing pathogens: *Legionella pneumophila, Mycobacterium avium,* and *Pseudomonas aeruginosa.* Environmental Health Perspectives 123: 749–758.
22. Johnson JS, Spakowicz DJ, Hong BY, Petersen LM, Demkowicz P, Chen L, Leopold SR, Hanson BM, Agresta HO, Gerstein M, Sodergren E, Weinstock GM (2019) Evaluation of 16S rRNA gene sequencing for species and strain-level microbiome analysis. Nat Commun 10:1–11.

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