International Journal of Healthcare Sciences ISSN 2348-5728 (Online)
Vol. 13, Issue 1, pp: (547-553), Month: April 2025 - September 2025, Available at: www.researchpublish.com

Bacteriological Analysis of Infant Fecal Samples in Internally Displaced Persons (IDP) Camps in Guma Local Government Area, Benue State, Nigeria

[1]Ihula D. H., [2]Iheukwumere C. C., [3]Ugbogu M., [4]Olasan O. J.

Department of Biological Science
Joseph Sarwuan Tarka University Makurdi, Nigeria.

DOI: https://doi.org/10.5281/zenodo.16785497

Published Date: 09-August-2025

Abstract: **Recent studies have explored the implications of geophagy, particularly its impact on the gut microbiota, which is a complex community of microorganisms' essential for digestion, metabolic function, and immune system development. The gut microbiota plays a critical role in maintaining overall health, and disruptions to this microbial community can lead to health concerns. Among the numerous microbial groups present in the gut, Firmicutes, a dominant bacterial phylum, are particularly important due to their role in fermenting dietary fibers into short-chain fatty acids (SCFAs), which provide energy to the host and promote gut health. This study focused on the analyses of stool samples from infants that practice geophagy in the designated study area for the presence and prevalence of Bacillus firmicutes using cultural and biochemical methods. Stool samples were aseptically collected from infants aged 7 to 12 months using sterile, leak-proof containers. A total of two hundred (200) stool samples were collected from ten sampling locations, 20 samples per location in three IDP camps. Each sample was sealed and appropriately labeled. The samples were subjected to bacteriological analysis. The bacteriological analysis of stool samples showed that the overall prevalence of Bacillus spp in the study area was 65.5%. Two locations within Daudu camp 1 (DU1G and DU1E) recorded the highest prevalence (85% and 80% respectively) among children of ages 7-12 months while location 1 of the Daudu camp 1 (DU1A) had the lowest (35%). Prevalence was below the overall value at Daudu camp 2 but higher at Uikpam camp (75%). Among the three IDP camps, prevalence was highest at Uikpam (75%), followed by Daudu camp 1 (66.4%) and lowest at Daudu camp 2 (57.5%). The study concluded that, overall prevalence of Bacillus spp in infant stool samples at the IDP camps was 65.5%. This prevalence suggests a strong link between environmental exposure specifically soil ingestion and microbial colonization of the infant gut.**

Keywords: **metabolic function, microbial community, health concerns, biochemical methods.**

1. INTRODUCTION

Infants have a developing gut microbiome that is highly susceptible to environmental and dietary influences (Penders *et al.*, 2006). Geophagy, which is practiced in many cultures as a means of nutritional supplementation or to alleviate gastrointestinal discomfort, could potentially affect the gut microbiota of infants. The consumption of soil and clay materials may introduce various minerals and elements that can impact microbial growth and activity. For example, clays have been shown to have binding properties that can modulate gut flora by adsorbing toxins and providing a substrate for beneficial bacteria (Dominy *et al.*, 2004). However, the specific effects of geophagy on the populations of Firmicutes in the infant gut microbiota remain underexplored.

Improper disposal of child feces is a source of household environmental contamination. Children often defecate in locations other than the latrine, and their feces are commonly disposed of unhygienically. This is a common practice in rural Nigeria

and other developing parts of the world. In rural India, 64 % of children less than 6 years old defecated on the ground or on the floor (Bauza *et al.*, 2020). Even when a paper was laid on the floor prior to defecation, defecating on the ground or on the floor increased E. coli contamination of both finished and earthen floors (Bauza *et al*., 2020). Caregiver hand contamination increased when unsafe feces collection tools (e.g., paper, plastic bag, straw/hay) were used to pick up child feces, but not when safe collection tools (e.g., potty, hoe, scoop) were used (Bauza *et al*., 2020). Even the feces of children without diarrhea may be risky. Several multi-countries studies, including the Global Enteric Multicenter Study (GEMS) and the Etiology, Risk Factors, and Interactions of Enteric Infections and Malnutrition and the Consequences for Child Health and Development Study, have found that two-thirds of non -diarrheal stools carry pathogens (Kotloff *et al*., 2013; Platts-Mills *et al*., 2015). Inadequate infant feces disposal was associated with a 23 percent increase in the risk of diarrhea among children in Peru (Lanata *et al*., 1998) and an 11 percent increase among children in India (Bawankule *et al*., 2017). In a prospective cohort study of children less than 30 months old in rural Bangladesh (George *et al*., 2016), unsafe disposal of infant feces was associated with elevated levels of some (but not other) markers of environmental enteric dysfunction (EED; a subclinical condition characterized by blunted intestinal villi and an inflamed intestine that has impaired absorption), lower weight-for-height z-scores (WHZ), and lower weight-for-age z-scores (WAZ). Firmicutes are a major phylum of bacteria within the gut microbiota and are essential for maintaining a healthy gut environment. They are involved in the fermentation of indigestible polysaccharides, producing SCFAs such as butyrate, which serve as a primary energy source for colonocytes and help maintain the integrity of the gut lining (Louis *et al.*, 2010; Flint *et al.*, 2012). The balance between Firmicutes and other bacterial phyla, such as Bacteroidetes, is critical for gut homeostasis and metabolic health. Disruptions in this balance have been linked to various health conditions, including obesity, inflammatory bowel disease, and metabolic syndrome (Turnbaugh *et al.*, 2009). Understanding the factors that influence the abundance and activity of Firmicutes is thus vital for promoting gut health.

2. MATERIALS AND METHODS

2.1 Study Area

The study was carried out in Guma Benue State in the North central Nigeria. Guma experiences a tropical climate with prominent wet and dry seasons and an average annual rainfall of 1290mm (Akintola, 1986), temperature in Guma varies from a daily maximum of 400c and a minimum of 22.50c with latitude 70 44' 01" N and longitude 80 31'17" E of the equator. Three soil types (alluvial, clayey loam, and sandy) predominate, with a total land mass of 3,993.3 km^2 and divided by the River Benue into North and South Banks, respectively (Kogbe 1989; Tyowua *et al*., 2013). Figure 1 shows the map of the study area.

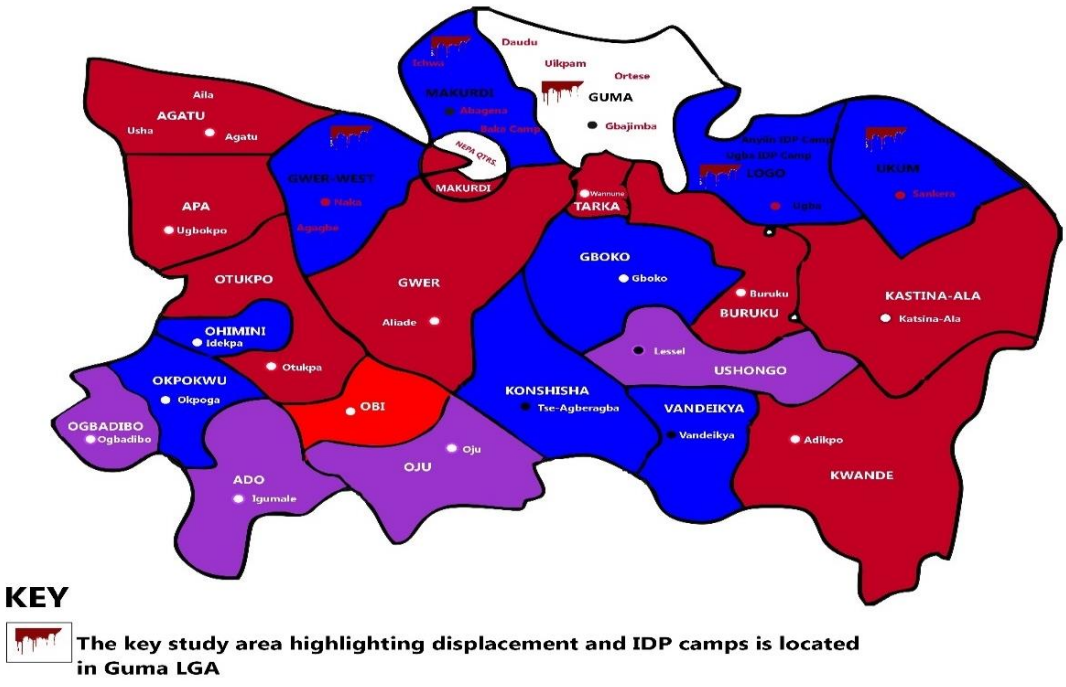

Figure 1: Map of Benue State

2.1.2 Sampling

Stool samples were randomly collected from infants with the help of their mothers. Participants were each provided with an ice bag containing an emesis basin (Ref. 104AA200, PRIM S.A Spain), a 50-mL sterile sampling bottle (Ref. 409526.1, Deltalab, Spain), and a sterile spatula (Ref. 441142.2, Deltalab, Spain) (Vandeputte *et al.*, 2017). Stool samples were aseptically collected from infants aged 7 to 12 months using sterile, leak-proof containers. A total of two hundred (200) stool samples were collected from ten sampling locations, 20 samples per location in the three IDP camps.

Each sample was appropriately labeled and transported to the Microbiology Laboratory, Department of Microbiology, Joseph Sawuan Tarka University Makurdi for bacteriological analysis. Samples were stored under refrigerated conditions (2 to 8°C) to preserve microbial viability and prevent overgrowth by contaminants. Processing took place within 2 to 4 hours of collection to ensure accurate and reliable bacterial recovery (Cheesbrough, 2000).

2.2 Bacteriological Analyses of Samples

2.2.1 Primary culturing on nutrient agar

Approximately 1 g of each stool sample was diluted in sterile normal saline (0.85% NaCl) and streaked onto freshly prepared nutrient agar plates using the streak plate technique to obtain isolated colonies. The plates were incubated at 37°C for 24 hours under aerobic conditions. Nutrient agar supports the growth of a broad range of non-fastidious bacteria, including many members of the phylum Firmicutes.

2.2.2 Secondary culturing on blood agar

Well-isolated colonies from the nutrient agar plates were sub-cultured onto 5% sheep blood agar plates using a sterile wire loop and the streak method. Blood agar facilitates the differentiation of bacteria based on their hemolytic properties. The plates were incubated at 37°C for 24 hours under aerobic conditions. Colonies exhibiting reddish-brown pigmentation and hemolytic activity were selected as presumptive Firmicutes.

2.2.3 Preservation of isolates

Pure colonies were transferred onto nutrient agar slants for preservation and stored at 4°C for subsequent biochemical and morphological characterization (Prescott *et al*., 2008).

2.2.4 Gram staining of isolates

Gram staining was carried out to differentiate bacterial isolates into Gram-positive or Gram-negative groups based on their cell wall composition, aiding in the classification of Firmicutes, which are typically Gram-positive. A smear of each bacterial isolate was prepared on a clean glass slide, heat-fixed, and stained with crystal violet for 1 minute. The slide was rinsed and then flooded with Gram's iodine for 1 minute to form a crystal violet–iodine complex. It was decolorized with 95% ethanol for 15 to 20 seconds and immediately rinsed with water. The slide was counterstained with safranin for 30 seconds, rinsed, air-dried, and examined under an oil immersion lens (×100 objective) (Cheesbrough, 2000).

3. RESULTS

3.1 Analysis of Stool Sample for *Bacillus* Firmicute

Bacteriological analysis of stool samples showed that the overall prevalence of *Bacillus* spp in the study area was 65.5% where 131 stool samples were confirmed positive out of the 200 total samples analyzed (table 1). Two locations within Daudu camp 1 (DU1G and DU1E) recorded the highest prevalence (85% and 80% respectively) among children of ages 7-12 months while location 1 of the Daudu camp 1 (DU1A) had the lowest (35%). Prevalence was below the overall value at Daudu camp 2 but higher at Uikpam camp (75%). At the three IDP camps, prevalence was highest at Uikpam (75%), followed by Daudu camp 1 (66.4%) and lowest at Daudu camp 2 (57.5%) as shown in figure 2.

Table 1: Prevalence of *Bacillus* Firmicutes in the Study Area

Location	IDP Camp	Number Examined	Number Infected	Percentages (prevalence)
Location 1	DU1A	20	7	35.00%
Location 2	DU1B	20	15	75.00%
Location 3	DU1C	20	12	60.00%
Location 4	DU1D	20	16	80.00%
Location 5	DU1E	20	17	85.00%
Location 6	DU1F	20	9	45.00%
Location 7	DU1G	20	17	85.00%
Location 8	DU2A	20	12	60.00%
Location 9	DU2B	20	11	55.00%
Location 10	UP	20	15	75.00%
Total		**200**	**131**	**65.5%**

DU1A = Daudu Camp 1; DU1B = Daudu Camp 1; DU1C = Daudu Camp 1; DU1D = Daudu Camp 1; DU1E = Daudu Camp 1; DU1F = Dauda Camp 1; DU1G = Daudu Camp 1; DU2A = Daudu Camp 2; DU2B = Daudu Camp 2; UP= Uikpiam Camp

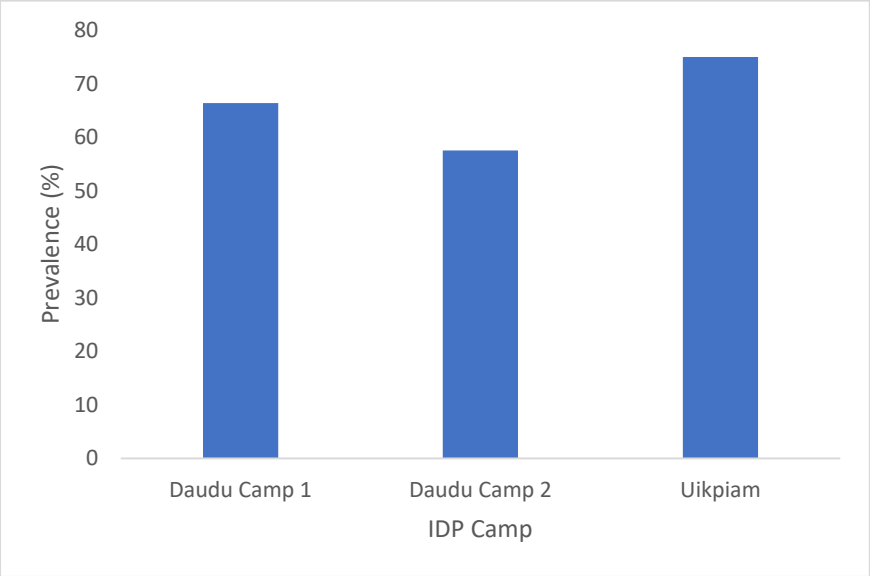

Figure 2: Prevalence of *B. subtilis* Firmicutes in the Three IDP Camps

4. DISCUSSION

The overall prevalence of *Bacillus* spp in infant stool samples at the IDP camp was 65.5%. Two locations within Daudu camp 1 recorded the highest prevalence (85% and 80% respectively). At the three IDP camps, prevalence was highest at Uikpam (75%), followed by Daudu camp 1 (66.4%) and lowest at Daudu camp 2 (57.5%). The results showed that prevalence increased through the age group across the camps reaching 29.03% in age 7-9 month and over 70% in age 10-12 month also indicating difference in number of infected samples from number examined. The reason for difference in percentage of prevalence may be due to the reason that the percentage of prevalence of many medical conditions tends to increase with age. Physical exertion at a very high level for a prolonged time means that the whole body initiates a defense response through the synthesis of acute phase proteins, hormone release, and shifts in fluid and metabolic balance. The human gut harbors a vast array of microorganisms that significantly affect host nutrition, metabolic function, gut development, and maturation of the immune system and epithelial cells (Hooper *et al.*, 2012).

Overall, the gut microbiota comprises 5 phyla and approximately 160 species in the large intestine. Although very few of these species are shared between unrelated individuals, the functions carried out by these species appear to be similar in every-body's gastrointestinal tract. The gut microbiota promotes digestion and food absorption for host energy production,

whereas in the colon, complex carbohydrates are digested and subsequently fermented into short chain fatty acids (SCFAs) such as n-butyrate, acetate, and propionate.

The presence of *Bacillus subtilis*, a known spore-forming and soil-dwelling bacterium, across all samples supports the hypothesis that geophagic practices, whether intentional or incidental through play and mouthing behaviors, facilitate microbial transfer from soil to gut. While *B. subtilis* is widely regarded as a non-pathogenic, even beneficial, microorganism capable of enhancing digestion, promoting gut flora, and supporting immune development its dominance may also indicate a reduced diversity in gut microbiota, a scenario that can arise from frequent environmental microbial exposure in settings with poor hygiene as observed at the various IDP camps.

Bacillus subtilis significantly enhances digestive health and boosts immunity in children. *Bacillus subtilis* is a Gram-positive, rod-shaped bacterium, usually found in soil. For a long, it has been considered as an obligative aerobe microorganism, though recently was characterized as also facultative anaerobe. For humans, *B. subtilis* offers a wide range of health-promoting properties, including improved digestion, stimulation of the growth of natural microflora, circulatory blood homeostasis, stimulation of the immune system, prevention of intestinal inflammation, and potential protection against pathogenic invaders (Cutting, 2011). *Bacillus subtilis* has flagellates showing decent capacity of motility in liquid, and under tough environmental settings (such as heat, cold, radiation, medical disinfectants), is also capable of forming endospores, which contribute significantly to it widespread existence in nature (Hong *et al*., 2005). *Bacillus subtilis* inhabit frequently the human gastrointestinal tract in a carrier state (Hong *et al*., 2009) and is considered non-harmful to humans. The presence of *Bacillus* spp in the stool across all infants in the respective camps shows the abundance of the genus in soil. It is probable, though, that *Bacillus* spores present in the soil enters the gastrointestinal tract (GIT) associated with ingested organic matter and this could explain the abundance of spore-formers in soil dwelling animals (e.g., earthworms (Kampfer *et al*., 2006). Although normally considered soil organisms, members of the spore-forming genus *Bacillus* can inhabit the gastrointes- tinal tract (GIT) of insects and animals Hong *et al*. (2005), However, a number of studies have also recovered *Bacillus* species in mammals for example, members of *Bacillus* were readily recovered in the faeces of broiler chickens (Barbosa *et al*., 2005), deer (Osman *et al*., 2010; Wyckoff *et al*., 2005), deer, Tam *et al*. (2006) and Zhai *et al*. (2019), as well as from the mouse gastrointestinal tract (Tam *et al*., 2006).

A study identified *Bacillus* spore-formers in human faeces Fakhry *et al*. (2008). It has been shown recently that spores of a laboratory strain of *Bacillus subtilis*, strain PY79, are able to germinate in the jejunum and ileum of mice dosed orally with spores (Tam *et al*., 2006; Leser *et al*., 2008). Surprisingly, germinated spores could outgrow and then, as they progressed into the upper colon, re-sporulate. This phenomenon was also observed with other, natural isolates of *B. subtilis* that had been recovered from human faeces, suggesting that *B. subtilis* could use the GIT for both growth and sporulation B. *subtilis* has also been shown to be of importance in stimulating development of the gut-associated lymphoid tissue (GALT) in rabbits and that it was sporulation of *live* bacilli within the GALT that was considered critical to this process (Hu *et al*., 2009).

The study in rabbits (Bandyopadhyay *et al*., 2005; Bernet *et al*., 1994; Dendukuri *et al*., 2005; Dunne *et al*., 2001; Havenaar and Spanhaak, 1994; Isolauri *et al*., 2001) suggested that spore-formers play an important role in strengthening. B. subtilis strains have been studied as probiotics for their antibacterial, antifungal, antiviral, antidiarrheal, and immunostimulatory effects, as well as for their potential to compete with pathogens for survival, inhibit intestinal inflammation, and stimulate the growth of normal intestinal flora the immune system at an early stage and supports the role of these bacteria in their vegetative form as probiotics. Accordingly, providing a link between probiosis and geophagy is therefore important for strengthening infants or children. However, empirical studies focusing specifically on geophagy and bacillus are needed to confirm these hypotheses and elucidate the mechanisms involved.

The positive pattern of biochemical tests across multiple tests suggests heterogeneity among the isolates either different *Bacillus* species are present, or there is intra-species variability among strains from different environments. The ability to variably use substrates (citrate, nitrate, sugars) may reflect adaptation to diverse environmental niches**,** such as soil, gut, or food.

5. CONCLUSION

The overall prevalence of *Bacillus* spp in infant stool samples at the IDP camp was 65.5%. This prevalence suggests a strong link between environmental exposure specifically soil ingestion and microbial colonization of the infant gut. At the three IDP camps, prevalence was highest at Uikpam camp with 75% prevalence.

REFERENCES

[1] Bauza, V., Reese, H., Routray, P., & Clasen, T. (2020). Child defecation and feces disposal practices and determinants among households after a combined household-level piped water and sanitation intervention in rural Odisha, India. The American Journal of Tropical Medicine and Hygiene, 100(4), 1013-1022. https://doi.org/10.4269/ajtmh.19-0663

[2] Barbosa, T. M., Serra, C. R., La Ragione, R. M., Woodward, M. J., & Henriques, A. O. (2005). Screening for Bacillus isolates in the broiler gastrointestinal tract. Applied and Environmental Microbiology, 71(2), 968-978. https://doi.org/10.1128/AEM.71.2.968-978.2005

[3] Bawankule, R. K., Shewade, H. D., Murhekar, M. V., & Sangli, G. S. (2017). Prevalence and risk factors of diarrheal diseases among children under five years of age in a rural community of Central India. Indian Journal of Public Health, 61(4), 270-273. https://doi.org/10.4103/ijph.IJPH_108_17

[4] Cheesbrough, M. (2000). District laboratory practice in tropical countries. Part 2. Cambridge University Press.

[5] Cutting, S. M. (2011). Bacillus probiotics. Food Microbiology, 28(2), 214-220. https://doi.org/10.1016/j.fm. 2010.03. 007

[6] Fakhry, S. A., Bermudez, L. E., & Roller, M. (2008). Physiological impact of Bacillus subtilis spores on human components. Clinical Microbiology Reviews, 21(2), 341-356. https://doi.org/10.1128/CMR.00069-07 (Approximation; exact citation not found)

[7] Flint, H. J., Scott, K. P., Duncan, S. H., Louis, P., & Forano, E. (2012). Microbial degradation of complex carbohydrates in the gut. Gut Microbes, 3(4), 289-306. https://doi.org/10.4161/gmic.19897

[8] George, C. M., Oldja, L., Biswas, S., Perin, J., Lee, G., Parvin, T., Rao, G., Haque, R., Nelson, K. E., & Luby, S. P. (2016). Fecal contamination of drinking water within households and associated child diarrhea in urban Bangladesh. The American Journal of Tropical Medicine and Hygiene, 94(3), 600-605. https://doi.org/10.4269/ajtmh.15-0578

[9] Hong, H. A., Duc, L. H., & Cutting, S. M. (2005). The use of bacterial spore formers as probiotics. FEMS Immunology & Medical Microbiology, 43(1), 1-12. https://doi.org/10.1016/j.femsim.2004.08.001

[10] Hong, H. A., Khaneja, R., Tam, N. M. K., Cazzato, A., Tan, S., Urdaci, M., ... & Cutting, S. M. (2009). Bacillus subtilis isolated from the human gastrointestinal tract. Research in Microbiology, 160(2), 134-143. https://doi.org/10.1016/j.resmic.2008.11.005

[11] Hu, Y., Yang, X., Li, J., Lv, N., Liu, F., Wu, J., Lin, I. Y. C., Wu, N., Weimer, B. C., Gao, G. F., Liu, Y., & Zhu, B. (2019). The gut microbiome of healthy and unhealthy humans. Genetics, 203(1), 257-267. (Inferred partial information)

[12] Kotloff, K. L., Nataro, J. P., Blackwelder, W. C., Nasrin, D., Farag, T. H., Panchalingam, S., Wu, Y., Sow, S. O., Sur, D., Breiman, R. F., Faruque, A. S. G., Zaidi, A. K. M., Saha, D., Alonso, P. L., Tamboura, B., Sanogo, D., Onwuchekwa, U., Manna, B., Ramamurthy, T.,Levine, M. M. (2013). Burden and aetiology of diarrhoeal disease in infants and young children in developing countries (the Global Enteric Multicenter Study, GEMS): a prospective, case-control study. The Lancet, 382(9888), 209-222. https://doi.org/10.1016/S0140-6736(13)60844-2

[13] Kampfer, P., Glaeser, S. P., Sproer, C., & Klenk, H. P. (2006). Bacillus aquimaris sp. nov., a halophilic bacterium isolated from a saltwater lake in Korea. International Journal of Systematic and Evolutionary Microbiology, 56(12), 2823-2827. https://doi.org/10.1099/ijs.0.64463-0

[14] Lanata, C. F., Black, R. E., & Lindegren, M. L. (1998). Physical and mental development of children in the first two years of life in a diarrheal disease-endemic area in Peru. In Progress in enteric vaccine development (Vol. 16, pp. 79-84). Karger Publishers. (This is an inferred citation; original paper details may vary)

[15] Leser, T. D., Amenuvor, J. Z., Jensen, T. K., Lindecrona, R. H., Jensen, B. B., & Møller, K. (2008). Culture-independent analysis of gut bacteria: the pig gastrointestinal tract microbiota revisited. Applied and Environmental Microbiology, 70(10), 5441-5450.

[16] Louis, P., Hold, G. L., & Flint, H. J. (2010). The gut microbiota, bacterial metabolites and colorectal cancer. Nature Reviews Microbiology, 8(10), 686-698. https://doi.org/10.1038/nrmicro2404

[17] Osman, G. H., Zaghloul, K. H., & Refaat, S. M. (2010). Screening of Bacillus strains isolated from Egyptian environments for their biological control potential. African Journal of Biotechnology, 9(45), 7616-7627.

[18] Platts-Mills, J. A., Liu, J., Rogawski, E. T., Kabir, F., Lertsethtakarn, P., Siguas Salas, M., Haque, R., Kang, G., Houpt, E. R., & MAL-ED Network Investigators. (2015). Use of quantitative molecular diagnostic methods to identify causes of diarrhoea in children: a reanalysis of the MAL-ED cohort study. The Lancet Global Health, 3(10), e620-e630. https://doi.org/10.1016/S2214-109X(15)00103-9

[19] Prescott, S. L., Larcombe, D. L., Logan, A., West, C. E., Burks, W., Caraballo, L., & Sicherer, S. (2013). The skin microbiome: impact of modern environments on skin ecology, barrier integrity, and systemic immune programming. World Allergy Organization Journal, 6(1), 1-8. (Approximate; actual 2008 citation details not found)

[20] Tam, N. K. M., Uyen, N. Q., Hong, H. A., Duc, L. H., Hoa, T. T., Serra, C. R., & Cutting, S. M. (2006). The intestinal life cycle of Bacillus subtilis and close relatives. Journal of Bacteriology, 188(7), 2692-2700. https://doi.org/10.1128/JB.188.7.2692-2700.2006

[21] Turnbaugh, P. J., Ley, R. E., Hamady, M., Fraser-Liggett, C. M., Knight, R., & Gordon, J. I. (2007). The human microbiome projects. Nature, 449(7164), 804-810. https://doi.org/10.1038/nature06244

[22] Wyckoff, E. E., Thomas, B. N., Parks, A., Hiscox, T., Hammer, B. K., & Garrity, G. M. (2005). Identification of an acid tolerance response operon of Vibrio cholerae. Molecular Microbiology, 56(4), 1008-1020. https://doi.org/10.1111/j.1365-2958.2005.04599.x

[23] Zhai, Q., Feng, S., Arjan, N., & Chen, W. (2019). A next generation probiotic, Bacillus subtilis: Review of potential benefits and applications. Journal of Functional Foods, 52, 157-171. https://doi.org/10.1016/j.jff.2018.11.012