Gut microbiota in hospitalized children with acute infective gastroenteritis caused by virus or bacteria in a regional Peruvian hospital

Andre Alonso Taco-Masias[1,*], Augusto R. Fernandez-Aristi[1,*], Angela Cornejo-Tapia[1], Miguel Angel Aguilar-Luis[1,2], Luis J. del Valle[3], Wilmer Silva-Caso[1,2], Victor Zavaleta-Gavidia[4], Pablo Weilg[5], Hernán Cornejo-Pacherres[4], Jorge Bazán-Mayra[4], Zully M. Puyen[6] and Juana del Valle-Mendoza[1,2]

[1] School of Medicine, Research and Innovation Centre of the Faculty of Health Sciences, Universidad Peruana de Ciencias Aplicadas, Lima, Peru
[2] Laboratorio de Biologia Molecular, Instituto de Investigación Nutricional, Lima, Peru
[3] Barcelona Research Center for Multiscale Science and Engineering, Departament d'Enginyeria Química, EEBE, Universitat Politècnica de Catalunya (UPC), Barcelona, Spain
[4] Dirección Regional de Salud de Cajamarca (DIRESA), Cajamarca, Peru
[5] Mercy One North Iowa Medical Center, Mason City, USA
[6] Centro Nacional de Salud Pública, Instituto Nacional de Salud, Lima, Peru
* These authors contributed equally to this work.

ABSTRACT

Background. Acute infective gastroenteritis (AIG) is a leading cause of mortality in children worldwide. In Peru, more than 40% of cases of AIG occurring in children under 5 years old. The disruption of the gut microbiota can increase risk for several health complications especially in patients with gastric infections caused by viruses or bacteria.

Objective. The main objective of this study was to describe the prevalence of 13 representative bacteria from the gut microbiota (GM) in stools samples from children under 5 years of age with acute infective gastroenteritis.

Results. The most commonly isolated bacteria from the GM were *Firmicutes* (63.2% 74/117) *Bacteriodetes* (62.4%; 73/117), *Lactobacillus* (59.8%; 70/117), *Prevotella* (57.2%; 67/117), *Proteobacterium* (53.8%; 63/117), regardless of the etiological agent responsible for the AIG. Interestingly, despite the high prevalence of *Firmicutes*, *Bacteroidetes*, *Lactobacillus* and *Prevotella* across all samples, a visible reduction of these agents was observed especially among patients with a single bacterial infection or even bacteria–bacteria coinfections when compared to viral etiologies. Patients with exclusive or mixed breastfeeding registered the highest amount of gut microbiota bacteria, in contrast to infants who received formula or were not breastfed.

INTRODUCTION

The intestinal microbiota is made up of various types of microorganisms, including more than 4,000 different species of bacteria (*Atarashi & Honda, 2011*). These microorganisms evolved alongside the host so that they play a fundamental role in human physiology by modulating normal intestinal functions and participating in local and systemic immune responses (*Chow et al., 2010*). A better understanding of the interactions between the microbiota and the host's immune system would help to understand the health and disease process that involves certain infectious and non-infectious pathologies such as inflammatory bowel disease (*Lin & Zhang, 2017*).

There are several factors that generate variations in the intestinal microbiota such as diet, geographical location, medication use, age, sex of the host, among others (*Brunkwall & Orho-Melander, 2017*; *Pushpanathan et al., 2019*). These variations can cause the loss of homeostasis between the intestinal microbiota and its surroundings, this is called dysbiosis, which is defined as the alteration in the composition and function of the microbiota. This condition has the ability to affect the permeability of the intestinal barrier, which causes inflammatory cascades, impaired mucosal integrity, among other harmful events at the intestinal level (*Robles-Alonso & Guarner, 2013*; *Icaza-Chávez, 2013*; *Giglio, Burgos & Cavagnari, 2013*; *Devaraj, Hemarajata & Versalovic, 2013*). In addition to this, several studies have described that alteration in the intestinal microbiota increases the risk of susceptibility to colonization with bacterial pathogens (*Nelson et al., 2012*).

Addressing the intestinal microbiota could represent a new approach to understand pathologies as frequent as infectious gastroenteritis, this because a significant reduction of the intestinal microbiota has been described in patients with gastroenteritis (*Nelson et al., 2012*; *Chen et al., 2017*).

In this context, acute gastroenteritis is characterized by the presence of diarrhea with or without vomiting and it is an important cause of morbidity and mortality in children. In general, the disease has a mild and self-limited clinical course, but in low-income countries, it represents a common cause of hospitalization (*Pieścik-Lech et al., 2013*; *Guarino et al., 2012*). It has been reported that acute gastroenteritis accounts for almost 2 million annual deaths in children under 5, and the highest mortality rates are concentrated in low-income countries (*Elliot, 2007*; *O'Ryan et al., 2014*). In Peru, a total of 119,417 episodes of acute gastroenteritis were reported in 2017, with more than 40% of cases in children under 5 years (*Ordoñez, 2018*).

Regarding gastroenteritis and its relationship with changes in the intestinal microbiota in patients under 5 years of age, the changes in the composition of the intestinal microbiota of patients with *Escherichia coli* (Shiga toxin –STEC) gastroenteritis producing have now been described, where be found a lower abundance of *Bifidobacterial* and *Clostridial* bacteria in infected infants (*Gigliucci et al., 2018*). Other studies in children with viral gastroenteritis describe microbiota changes in children with severe infectious gastroenteritis with greater abundance of *Prevotellaceae, Staphylococcaceae and Coriobacteriaceae* compared to healthy children (*Chen et al., 2017*).

Increasing evidence suggests that manipulation of the intestinal microbiota could treat or even prevent some intestinal diseases (*Gigliucci et al., 2018*; *Sommer et al., 2017*).

This is why the main objective of this study was to describe the prevalence of 13 bacteria representative of the intestinal microbiota in stool samples of children under 5 years of age with acute infective gastroenteritis (AIG).

MATERIAL AND METHODS

Patients and samples

A secondary analysis was performed on stools samples from a cross-sectional study in children under 5 years of age hospitalized due to an acute infective gastroenteritis (AIG) at *Hospital Docente Regional de Cajamarca* in rural Northern Peru (*Cornejo-Tapia et al., 2017*).

The original study enrolled 117 hospitalized children with AIG from January 2010 to December 2012 for etiological identification in stool samples of the following pathogens: Rotavirus, Adenovirus, Norovirus, *C. jejuni*, *C. coli*, *Shigella*, *Salmonella*, Enteroaggregative *E. coli* (EAEC) and Enteropathogenic *E. coli* (EPEC) (*Cornejo-Tapia et al., 2017*).

The diagnosis of AIG was defined as diarrhea lasting less than 14 days along with symptoms and signs such as fever, nausea, vomiting, and dehydration based on the guidelines' criteria of the European Society for Pediatric Infectious Diseases. Nutritional status and dehydration status were based on the World Health Organization's criteria using Z-scores (weight for age).

PCR was used to detect 13 common gut microbiota (GM) bacterial genera including *Veillonella*, *Bacteroides*, *Bacteriodetes*, *Fusobacterium*, *Lactobacillus*, *Firmicutes*, *Actinobacterium*, *Bifidobacterium*, *Eubacterium*, *Prevotella*, *Enterococcus*, *Proteobacterium*, *Clostridium*.

Ethics statement

This study was approved by the Ethics Committee from *Universidad Peruana de Ciencias Aplicadas* in Lima, Peru (Document No: FCS/291-12-17). The samples were obtained in the context of the epidemiological/syndromic surveillance program according to the health directives of the National Center for Epidemiology, Disease Control Prevention of the Ministry of Health of Peru. In this way, the collection of samples was exempt of informed consent.

Samples

The samples from the original study were stored at −20 °C at the laboratory of the Research Center e Innovation for Health Sciences, Universidad Peruana de Ciencias Aplicadas (Lima, Peru).

Nucleic acid extraction (DNA)

DNA extraction was performed from 200 μL of the fecal suspensions using a High Pure PCR template preparation kit (Roche Applied Science, Mannheim, Germany) performed according to the kit's instructions.

PCR amplification for detection gut microbiota

The 13 bacteria from the gut microbiota were amplified using nucleic acid extracted from fecal samples. Specific primers targeting different bacterial genera were used to characterize the fecal microbiota, these were previously described by *Murri et al. (2013)*. Amplifications were initiated with an incubation at 95 °C for 2 min, followed by 40 cycles of 95 °C for 30 s, 58 °C for 30 s, and 72 °C for 30 s; with a final extension at 72 °C for 5 min. Amplified products were visualized on a 2.5% agarose gel containing 3 µg/mL of ethidium bromide in 1x Tris-borate buffer and photographed under ultraviolet illumination (UV Transilluminator KODAC LOGIC 1500, New Haven, USA). Amplified products were recovered from the gel, purified (SpinPrep^{TM} Gel DNA Kit, San Diego, USA) and sent for commercial sequencing service (Macrogen, Seoul, Korea).

Data analysis

Quantitative variables were described as frequencies and percentages for each group. The frequency distribution between groups was analyzed by χ^2-Test of homogeneity using MiniTab v18.1 software (MiniTab Inc., USA). The authors could not get more data information such as type of delivery, daycare attendance, vaccines, diet and genetic factors; due to that this study in its objective original did not focus on these variables.

RESULTS

A total of 117 samples from patients under 5 years old with AIG were analyzed via PCR to detect 13 bacteria from the gut microbiota (GM). In the population studied, infants younger than 12 months-old were the most common age group in 36.7% (43/117) followed by children older than 18 months old in 32.5% (38/117) and children 12–17 months in 23.9% (28/117), however there is a group of children of unknown age in 6.8% (8/117). Mixed breastfeeding was the most frequent practice in 39.3% (46/117), followed by exclusive breastfeeding in 29.1% (34/117). The attending physicians registered clinical symptoms at admission. Nausea and vomits were the most common complaint in 59% of patients (69/117), followed closely by fever in 54.7% (64/117). The distribution of each pathogen according to age, shows that the most predominant pathogens are rotaviruses followed by shigella, followed by adenovirus (Fig. 1).

In general, the distribution of GM for different types of gastrointestinal infection (e.g., virus, bacteria or their coinfections) were similar or showed no significant differences between them (χ^2-Test, $p > 0.05$) (Fig. 2). However, the most commonly isolated bacteria from the GM were *Firmicutes* (74/117), *Bacteriodetes* (73/117), *Lactobacillus* (70/117), *Prevotella* (67/117), *Proteobacterium* (63/117). These five bacteria were also the most frequently isolated in all samples regardless of their etiological agents isolated (Table 1). Interestingly, despite the high prevalence of *Firmicutes*, *Bacteroidetes*, *Lactobacillus*, and *Prevotella* across all samples, a visible reduction of these agents was observed especially among patients with a single bacterial infection or even bacteria –bacteria coinfections when compared to viral etiologies (Fig. 2) (Table S1).

Finally, gut microbiota variations were also studied among the several types of breastfeeding practices in the children with an acute gastroenteritis. Patients with exclusive
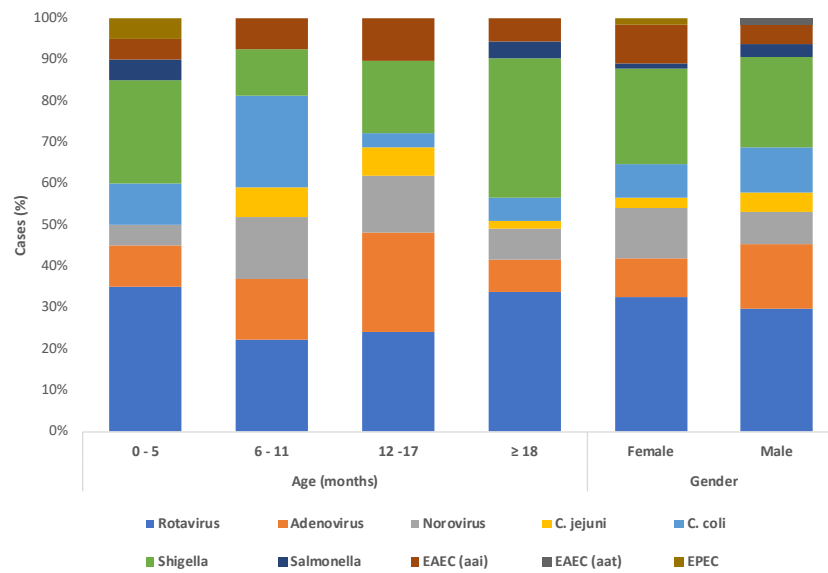
Figure 1 Demographics and etiological detection in patients with acute gastroenteritis.
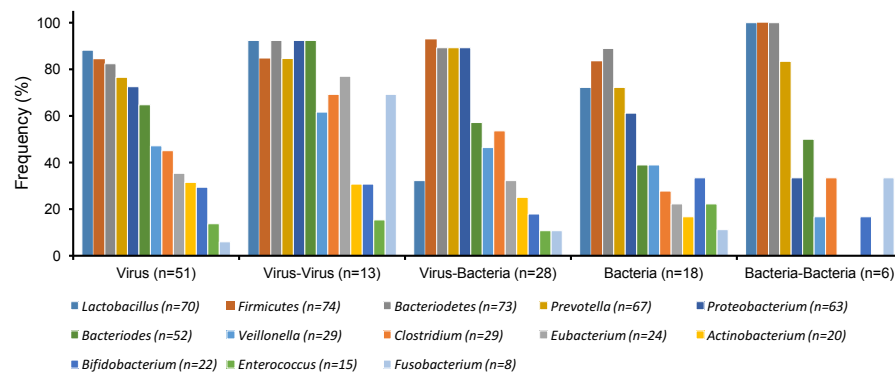Full-size ⊡ DOI: 10.7717/peerj.9964/fig-1

Figure 2 Gut microbiota detected in patients with single etiologies vs co-infections (χ^2 test, $p > 0.05$).
Full-size ⊡ DOI: 10.7717/peerj.9964/fig-2

or mixed breastfeeding registered the highest amount of gut microbiota bacteria, in contrast to infants who received formula or were not breastfed (χ^2-Test, $p < 0.05$) (Fig. 3). In addition, exclusive and mixed breastfeeding were significantly different (χ^2-Test, $p < 0.05$).

DISCUSSION

The gut microbiota (GM) is a complex community of microorganisms inhabit in the human gastrointestinal tract. This collection of bacteria, archaea, and eukarya offer benefits to the host strengthening gut integrity, protecting against potential pathogens and regulating host immunity (*Van den Elsen et al., 2017*; *Goulet, 2015*; *Petrof et al., 2013*; *Munoz et al., 2013*; *Human Microbiome Project C, 2012*; *Martz et al., 2014*; *Tremaroli & Bäckhed, 2012*; *Schippa*

Table 1 Gut microbiota detected in patients with viral and bacterial acute gastroenteritis (χ^2 -Test, $p > 0.05$).

Gut Microbiota	VIRUS			BACTERIA						
	Rotavirus (n = 43)	Adenovirus (n = 17)	Norovirus (n = 14)	C. jejuni (n = 5)	C. coli (n = 13)	Shiguella (n = 31)	Salmonella (n = 3)	EAEC		EPEC
								aai (n = 10)	aat (n = 1)	eae (n = 1)
Firmicutes (n = 74)	37/43	16 /17	9/14	4/5	12/13	29/31	3/3	10/10	1/1	1/1
Bacteriodetes (n = 73)	37/43	15/17	11/14	4/5	11/13	29/31	3/3	10/10	1/1	1/1
Lactobacillus (n = 70)	39/43	14/17	13/14	4/5	13/13	26/31	3/3	9/10	1 /1	1/1
Prevotella (n = 67)	34/43	14/17	10/14	3 /5	10/13	26/31	3/3	10/10	1/1	1/1
Proteobacterium (n = 63)	35/43	12/17	11/14	4/5	13/13	24/31	3/3	8/10	1/1	1/1
Bacteriodes (n = 52)	28/43	13/17	12/14	2/5	6/13	17/31	2/3	7/10	0/1	0/1
Clostridium (n = 29)	23/43	6/17	9/14	1/5	2/13	1/31	2/3	7 /10	1/7	0/1
Veillonella (n = 29)	22/43	15/17	6/14	2/5	4/13	1/31	1/3	6/10	1/1	1/1
Eubacterium (n = 24)	16/43	6/17	8/14	1/5	2/13	5/31	1/3	4/10	1/1	0 /1
Bifidobacterium (n = 22)	12/43	2/17	5/14	2/5	3/13	4/31	0/3	3 /10	1/1	1/1
Actinobacterium (n = 20)	11/43	6/17	3/14	1/5	4/13	3/31	0/3	1/10	1/1	0/1
Enterococcus (n = 15)	5 /43	4/17	2/14	0/5	3/13	2/31	1/3	0/10	0/1	1/1
Fusobacterium (n = 8)	4/43	0 /17	1 /14	0/5	1/13	4/31	1/3	1/10	0/1	0/1

& Pia-Conte, 2014). However, alterations in the microbiota composition (dysbiosis) can result in the disruption of these protective mechanisms increasing their risk for infections (Van den Elsen et al., 2017; Goulet, 2015; Thursby & Juge, 2017).

The most common cause of AIG in children is viruses in approximately 70% of cases, followed by bacteria in 10–20% of patients (Elliot, 2007; Chen et al., 2006). Among the viral etiologies, Rotavirus is the most frequent pathogen with different viral strains varying by season and geographies and affecting primarily children under 5 years old (Elliot, 2007; Chen et al., 2006; Shah et al., 2017). However, in developing countries, pathogenic bacteria can be the leading cause of AIG with E. coli being the most commonly isolated etiology followed by Shigella flexneri (Elliot, 2007; Dedwal, Pol & Bharadwaj, 2016; Kotloff et al., 2013). In this study, a viral etiology was isolated in 63.2% of samples and bacteria were detected in 54.7%. Rotavirus was the most common etiology detected in 36.8% (43/117) of samples, followed by Shigella in 26.5% (31/117).
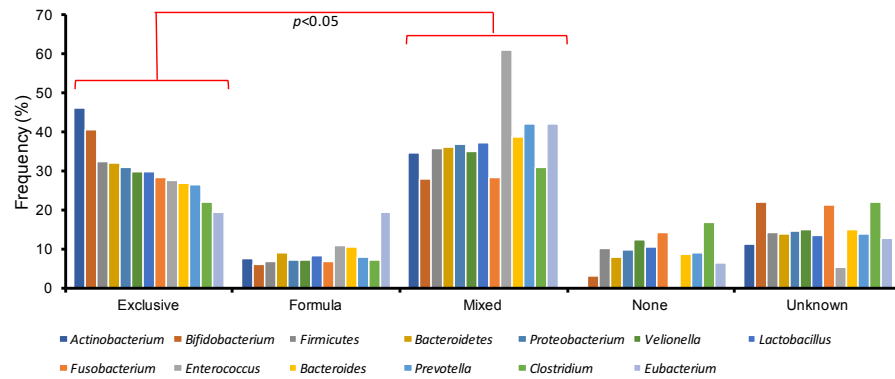
Figure 3 Gut microbiota variations among the different types of breastfeeding in children with acute gastroenteritis.

Full-size ⬚ DOI: 10.7717/peerj.9964/fig-3

Undernutrition in children is associated with a reduced microbiota diversity, characterized by a low prevalence of *Bacteroidetes* and a high number of *Proteobacteria*. This susceptible population has a higher infection risk for *Klebsiella* and *Escherichia* (*Pekmez, Dragsted & Brahe, 2018*; *Monira et al., 2011*; *Goday, 0000*; *Chiejina & Samant, 2018*). Moreover, a study in India found that infections due to *Escherichia*, *Streptococcus*, *Shigella*, *Enterobacter* and *Veillonella* generate are more frequent in children with a malnutrition (*Pekmez, Dragsted & Brahe, 2018*). In this study, only 14.5% (17/117) of patients had chronic malnutrition as a risk factor for acute gastroenteritis and only 4 cases of Shigellosis and 3 cases of EAEC were observed. Moreover, *Proteobacterium* were less common among patient with a bacterial AIG.

Studies have shown that breastfeeding provides prebiotics and bacterial communities that stabilize the GM during the first year of life, including *Bifidobacterium*, *Lactobacillus* and *Bacteriodetes* (*Yu et al., 2013*; *Bäckhed et al., 2015*). This beneficial effect has been reported in breastfed and mixed-fed infants who acquire a greater amount of bacteria from the human milk compared to their counterparts (*Monira et al., 2011*). The most patients had mixed feeding (39.3%), followed by those who received exclusive breastfeeding (29.1%). The GM bacteria in those feeding patterns that included human milk presented a higher frequency of detection especially in the *Bifidobacterium*, *Actinobacterium and Enteroccus* genera. This finding further supports the study of *Bäckhed et al. (2015)* who showed that the sole only presence of human milk predisposes to greater GM diversity, compared with the population that was not breastfed. However, we must stand out there are some bacteria that need being supported by human milk or formula such as *Enterococcus* and *Actinobacteirum*, that were undetectable in people that received none, as we can note in this study.

The most prevalent microbiota bacteria were *Firmicutes* (63.2%), *Bacteriodetes* (62.4%), *Lactobacillus* (59.8%), *Prevotella* (57.3%), *Proteobacterium* (53.8%) and *Bacteroides* (44.4%). On the contrary, *Clostridium* (24.8%), *Veillonella* (24.8%), *Eubacterium* (20.5%), *Bifidobacterium* (18.8%), *Actinobacterium* (17.1%), *Enterococcus* (12.8%) and

Fusobacterium (6.8%) were the least common detected. These last three can guide us that the simple presence of any etiological agent, whether viral or bacterial, it can be disruptive for these GM bacteria. That is why those who can generate any alteration in their homeostasis are principally the cytotoxic bacteria such as *Shigella* and *E. coli* species. Such is so, the low detection of *Enterococcus, Bifidobacterium* and *Fusobacterium* is due to the presence of *Shigella flexneri* and EAEC. However, we shall state that some viral aetiologies, such as double stranded linear DNA virus, can be dangerous for Enterococcus due to these virus, specially *Adenovirus*, can disturb the intestinal pH generating an acid pH leading to death of *Enterococcus,* that lives in alkaline pH (*Rexroad et al., 2016*). As stated above, the decrease of GM communities are correlated with chronic disease due to the persistent inflammatory state created by dysbiosis (*Kowalska-Duplaga et al., 2019*). Many of these ones are autoimmune diseases and they are linked to this trend such as Crohn's disease in which the pivot GM finding is the decreased *Bifidobacterium* and *Fusobacterium* (*Pittayanon et al., 2019*). In contrast to ulcerative colitis that has the same pattern of GM, but with a more predominant decrease in *Fusobacterium* (*Wang et al., 2018*). On the other hand, there are other contexts, such as Henoch-Schönlein purpura where the keystone is the detection of *Enterococcus* who play a role in the pro-inflammatory state described above (*Gosh et al., 2014*). Although this study has used a qualitative assay, we have observed the absence of these GM communities. We are also able to hypothesis that the lack of those specific bacterial communities exerts the pro-inflammatory states in chronic diseases and may play a role as protective GM bacteria. However, it is difficult to speculate what these frequencies implies in these patients since this is a new research field. Nonetheless, based on currently published studies on these bacteria microbiota different hypotheses can be proposed.

The human GM contains at least 1000 different species of known bacteria, protective mechanisms against AIG have been described in only a few of them. For example, *Bacteroides*, which is the most predominant anaerobic bacteria in the human gastrointestinal tract (HGIT), provide sugar molecules through carbohydrate fermentation which are important in the activation of T-cell-dependent immune response, and the expression of protective proteins from the Paneth cell (defensins and Ang4) (*Wexler, 2007*). *Proteobacterium*, play an important role in the transition from the neonatal to adult microbiota, favoring colonization by obligate anaerobes; which later are replaced by *Firmicutes* and *Bacteroidetes*. These bacteria were commonly detected in these patients, with no significant difference in their prevalence across the different etiologies isolated. However, an increased number of *Firmicutes* and *Bacteroidetes* in the gut can be used as an indicator of dysbiosis (*Shin, Whon & Bae, 2015*; *Ramakrishna, 2013*).

Other important bacteria are *Lactobacillus*, which are a major probiotic factor for other bacteria, as well as a modifier of the cell surface glycan, enhancing the action of *Bacteroidetes* (*Schippa & Pia-Conte, 2014*). Additionally, *Lactobacillus* serves as a microbiota stabilizer between other communities exchanging DNA traits and other soluble factors (*Szajewska et al., 2013*). *Lactobacillus* was common in these patients and some strains, such as *Lactobacillus* GG may reduce the duration of diarrhea in AIG (*Dubin & Pamer, 2014*).

Enterococcus contributes to colonic homeostasis through PPARγ1-induced IL-10 and TGF-ßexpression and can reduce the severity of infectious diarrhea in children (*Odamaki et al., 2016*). Furthermore, *Enterococcus* is used as a probiotic, as well as part of the fecal microbiota transplant along with *Lactobacillus casei* (*Odamaki et al., 2016*). In patients' samples, *Enterococcus* was less commonly isolated, especially in the patients with shigellosis in which an inflammatory destruction of the intestinal barrier is usually observed.

Bifidobacterium its main role is to down-regulate pro-inflammatory responses in the gut epithelium and *Clostridium* spp. that provides substrates from products of fermentation acting as an impressive anti-inflammatory agent by avoiding the activation of the transcription factor, NF-κB (*Lopetuso et al., 2013*; *Sun & Kato, 2016*). Mainly, both bacteria were not detected in this study. *Veillonella* and *Fusobacterium* were expected to be less commonly isolated as both have a preference for oral cavity, working as a bridge organism between early and late colonizers (*Sun & Kato, 2016*).

This study is among the first ones to describe the different prevalence of the most important bacteria from the GM in patients with multiple etiologies of AIG. However, as we have discussed previously it is difficult to hypothesize what these frequencies implies in the patient due to the study limitations.

The first study limitation was the lack of normal range values to determine if the number of bacteria from the GM we isolated are decreased or augmented. Nonetheless, we tried to compare these findings with GM bacteria known to be more predominant in different regions of the gastrointestinal tract. Second, since the only patient with AIG were studied it is not possible to compare this prevalence with a normal equivalent population. Finally, although interesting findings such as the visible reduction of GM bacteria among patients with a bacterial infection or the higher number of GM in a patient receiving exclusive or mixed breastfeeding were worth mentioning; to imply a cause–effect relationship is not possible.

CONCLUSION

In conclusion, despite the etiology of the infection, the most frequent bacteria identified were *Firmicutes*, *Bacteroidetes*, *Lactobacillus*, *Prevotella*, and *Proteobacterium*. However, there are some important bacteria which has a crucial role maintaining the integrity of the GM and the enteric epithelium among all described GM bacteria, such as *Bacteroidetes*, *Enterococcus*, *Bifidobacterium* and *Actinobacterium*, whose the main role is stabilize the GM. It is very important to note that the microorganisms most affected are the ones who promote the homeostasis and the continuity of the intestinal microbiome. In addition, we have to take into account the *Actinobacterium* and the *Bifidobacterium* whose main role falls in the modulation of gut permeability and the main axis, which play a crucial role when there is a pro-inflammatory state, either an infection or a systemic disease, promoting a protective barrier in the mucosa, and stabilizing the GM along with *Bacteroidetes*.

These data guide us that every GM bacteria has an important role to maintain the homeostasis. For all of above, we can conclude that the current treatment regimens must focus on a symbiotic therapy, which content more gut microbiota bacteria instead of the

single regimen, such as *Lactobacillus* regimens, which are the most used ones in the AIGs, since the bacteria of the intestinal microbiota are distributed in a same way in viral, bacterial gastroenteritis or in various coinfections.

ACKNOWLEDGEMENTS

We thank the staff of the health network from DIRESA Cajamarca, Peru.

ADDITIONAL INFORMATION AND DECLARATIONS

Competing Interests

The authors declare there are no competing interests.

Author Contributions

- Andre Alonso Taco-Masias and Augusto R. Fernandez-Aristi conceived and designed the experiments, analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the paper, and approved the final draft.
- Angela Cornejo-Tapia, Miguel Angel Aguilar-Luis and Wilmer Silva-Caso conceived and designed the experiments, performed the experiments, analyzed the data, authored or reviewed drafts of the paper, and approved the final draft.
- Luis J. del Valle and Zully M. Puyen analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the paper, and approved the final draft.
- Victor Zavaleta-Gavidia, Hernán Cornejo-Pacherres and Jorge Bazán-Mayra conceived and designed the experiments, authored or reviewed drafts of the paper, collections samples and data, and approved the final draft.
- Pablo Weilg performed the experiments, authored or reviewed drafts of the paper, and approved the final draft.
- Juana del Valle-Mendoza conceived and designed the experiments, performed the experiments, analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the paper, and approved the final draft.

Human Ethics

The following information was supplied relating to ethical approvals (i.e., approving body and any reference numbers):

This study was approved by the Ethics Committee from Universidad Peruana de Ciencias Aplicadas in Lima, Peru (Ethical application Ref: FCS/291-12-17). The samples were obtained in the context of the epidemiological/syndromic surveillance program according to the health directives of the National Center for Epidemiology, Disease Control Prevention of the Ministry of Health of Peru. In this way, the collection of samples was exempt of informed consent.

Data Availability

The following information was supplied regarding data availability:

The raw data is available at Figshare: Del Valle Mendoza, Juana; Taco-Masias, André; Fernández-Aristi, Rafael; Tapia, Angela Cornejo; Weilg, Pablo; Zavaleta-Gavidia, Victor; et al. (2020): Dataset Microbiota AG y2020m03. figshare. Dataset. https://doi.org/10.6084/m9.figshare.12009492.v1.

Supplemental Information

Supplemental information for this article can be found online at http://dx.doi.org/10.7717/peerj.9964#supplemental-information.

REFERENCES

Atarashi K, Honda K. 2011. Microbiota in autoimmunity and tolerance. *Current Opinion in Immunology* **23**:761–768 DOI 10.1016/j.coi.2011.11.002.

Bäckhed F, Roswall J, Peng Y, Feng Q, Jia H, Kovatcheva-Datchary P, Li Y, Xia Y, Xie H, Zhong H, Khan MT, Zhang J, Li J, Xiao L, Al-Aama J, Zhang D, Lee YS, Kotowska D, Colding C, Tremaroli V, Yin Y, Bergman S, Xu X, Madsen L, Kristiansen K, Dahlgren J, Wang J. 2015. Dynamics and stabilization of the human gut microbiome during the first year of life. *Cell Host & Microbe* **17(5)**:690–703 DOI 10.1016/j.chom.2015.04.004.

Brunkwall LY, Orho-Melander M. 2017. The gut microbiome as a target for prevention and treatment of hyperglycaemia in type 2 diabetes: from current human evidence to future possibilities. *Diabetologia* **60**:943–951 DOI 10.1007/s00125-017-4278-3.

Chen S, Ni YH, Chen HL, Chang MH. 2006. Microbial etiology of acute gastroenteritis in hospitalized children in Taiwan. *Journal of the Formosan Medical Association* **105(12)**:964–970 DOI 10.1016/S0929-6646(09)60280-1.

Chen SY, Tsai CN, Lee YS, Lin CY, Huang KY, Chao HC, Lai MW, Chiu CH. 2017. Intestinal microbiome in children with severe and complicated acute viral gastroenteritis. *Scientific Reports* **7**:46130 DOI 10.1038/srep46130.

Chiejina M, Samant H. 2018. Diarrhea, and Viral. [Updated 2017 Dec 5]. In: *StatPearls [Internet]*. Treasure Island: StatPearls Publishing *Available at https://www.ncbi.nlm.nih.gov/books/NBK470525/*.

Chow J, Lee SM, Shen Y, Khosravi A, Mazmanian SK. 2010. Host–bacterial symbiosis in health and disease. *Advances in Immunology* **107**:243–274 DOI 10.1016/B978-0-12-381300-8.00008-3.

Cornejo-Tapia A, Orellana-Peralta F, Weilg P, Bazan-Mayra J, Cornejo-Pacherres H, Ulloa-Urizar G, Aguilar-Luis MA, Pons MJ, Del Valle Mendoza J. 2017. Etiology, epidemiology and clinical characteristics of acute diarrhea in hospitalized children in rural Peru. *The Journal of Infection in Developing Countries* **11**(**11**):826–832 DOI 10.3855/jidc.7881.

Dedwal A, Pol S, Bharadwaj R. 2016. Microbial etiology of acute gastroenteritis in pediatric patients in Western India [Abstract 1206]. *Open Forum Infectious Diseases* **3**(**suppl_1**) DOI 10.1093/ofid/ofw172.909.

Devaraj S, Hemarajata P, Versalovic J. 2013. La microbiota intestinal humana y el metabolismo corporal: implicaciones con la obesidad y la diabetes. *Acta Bioquím Clín Latinoam* **47**(**2**):421–434.

Dubin C, Pamer E. 2014. Enterococci and their interactions with the intestinal microbiome. *Microbiology Spectrum* **5**(**6**):1–24.

Elliot EJ. 2007. Acute gastroenteritis in children. *BMJ* **334**(**7583**):35–40 DOI 10.1136/bmj.39036.406169.80.

Giglio ND, Burgos F, Cavagnari BM. 2013. Microbiota intestinal: sus repercusiones clínicas en el cuerpo humano. *Arch Argent Pediatría* **111**(**6**):523–527.

Gigliucci F, Von Meijenfeldt FA, Knijn A, Michelacci V, Scavia G, Minelli F, Dutilh BE, Ahmad HM, Raangs GC, Friedrich AW, Rossen JWA, Morabito S. 2018. Metagenomic characterization of the human intestinal microbiota in fecal samples from STEC-infected patients. *Frontiers in Cellular and Infection Microbiology* **8**:25 DOI 10.3389/fcimb.2018.00025.

Goday PMBBS. Malnutrition in children in resource-limited countries: clinical assessment. In: Post TW, ed.UpToDate. Waltham: UpToDate Inc *Available at http://www.uptodate.com* (accessed on 31 March 2018).

Gosh TS, Gupta SS, Bhattacharya T, Yadav D, Barik A, Chowdhury A, Das B, Mande SS, Nair GB. 2014. Gut microbiomes of Indian children of varying nutritional status. *PLOS ONE* **9**:e95547 DOI 10.1371/journal.pone.0095547.

Goulet O. 2015. Potential role of the intestinal microbiota in programming health and disease. *Nutrition Reviews* **73**(**Suppl 1**):32–40 DOI 10.1093/nutrit/nuv039.

Guarino A, Dupont C, Gorelov AV, Gottrand F, Lee JK, Lin Z, Lo Vecchio A, Nguyen TD, Salazar-Lindo E. 2012. The management of acute diarrhea in children in developed and developing areas: from evidence base to clinical practice. *Expert Opinion on Pharmacotherapy* **13**:17–26 DOI 10.1517/14656566.2011.634800.

Human Microbiome Project C. 2012. Structure function and diversity of the healthy human microbiome. *Nature* **486**:207–214 DOI 10.1038/nature11234.

Icaza-Chávez M. 2013. Microbiota en la salud y la enfermedad. *Revista de Gastroenterología de México* **78**(**4**):240–248 DOI 10.1016/j.rgmx.2013.04.004.

Kotloff KL, Nataro JP, Blackwelder WC, Nasrin D, Farag TH, Panchalingam S, Wu Y, Sow SO, Sur D, Breiman RF, Faruque AS, Zaidi AK, Saha D, Alonso PL, Tamboura B, Sanogo D, Onwuchekwa U, Manna B, Ramamurthy T, Kanungo S, Ochieng JB, Omore R, Oundo JO, Hossain A, Das SK, Ahmed S, Qureshi S, Quadri F, Adegbola RA, Antonio M, Hossain MJ, Akinsola A, Mandomando I, Nhampossa T, Acácio S,

Biswas K, O'Reilly CE, Mintz ED, Berkeley LY, Muhsen K, Sommerfelt H, Robins-Browne RM, Levine MM. 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. *Lancet* **382**(**9888**):209–222 DOI 10.1016/S0140-6736(13)60844-2.

Kowalska-Duplaga K, Gosiewski T, Kapusta P, Sroka-Oleksiak A, Wędrychow-icz A, Pieczarkowski S, Ludwig-Słomczyńska AH, Wołkow PP, Fyderek K. 2019. Diferences in the intestinal microbiome of healthy children and patients with newly diagnosed Crohn's disease. *Scientific Reports* **9**(**1**):18880 DOI 10.1038/s41598-019-55290-9.

Lin L, Zhang J. 2017. Role of intestinal microbiota and metabolites on gut homeostasis and human diseases. *BMC Immunology* **18**(**1**):2 DOI 10.1186/s12865-016-0187-3.

Lopetuso L, Scaldferri F, Petito V, Gasbarrini A. 2013. Commensal Clostridia: leading players in the maintenance of gut homeostasis. *Gut Pathogens* **5**:23 DOI 10.1186/1757-4749-5-23.

Martz S-LE, McDonald JAK, Sun J, Zhang Y-G, Yhurist-Doutsch S, Arrieta M, Vogt SND, Finlay B. 2014. Gastrointestinal microbiota-mediated control of enteric pathogens. *Annual Review of Genetics* **48**:361–382 DOI 10.1146/annurev-genet-120213-092421.

Monira S, Nakamura S, Gotoh K, Izutsu K, Watanabe H, Alam NH, Endtz HP, Cravioto A, Ali SI, Nakaya T, Horii T, Iida T, Alam M. 2011. Gut microbiota of healthy and malnourished children in Bangladesh. *Frontiers in Microbiology* **2**:228 DOI 10.3389/fmicb.2011.00228.

Munoz S, Martz S, He S, Noordhof C. 2013. Tu1982 investigating the therapeutic potential of Met-1 probiotic mixture in an infectious vs. chemically-induced model of acute colitis in mice. *Gastroenterology* **144**(**5**):S-896.

Murri M, Leiva I, Gomez-Zumaquero JM, Tinahones FJ, Cardona F, Soriguer F, Queipo Ortuño MI. 2013. Gut microbiota in children with type 1 diabetes differs from that in healthy children: a case-control study. *BMC Medicine* **11**:46 DOI 10.1186/1741-7015-11-46.

Nelson A, Walk S, Taube S, Taniuchi M, Houpi E, Wobus C, Young VB. 2012. Disruption of the human gut microbiota following Norovirus infection. *PLOS ONE* **7**(**10**):e48224 DOI 10.1371/journal.pone.0048224.

Odamaki T, Kato K, Sugahara H, Hashikura N, Takahashi S, Xiao JZ, Abe F, Osawa R. 2016. Age-related changes in gut microbiota composition from newborn to centenarian: a cross-sectional study. *BMC Microbiology* **16**:90 DOI 10.1186/s12866-016-0708-5.

Ordoñez L. 2018. Situacion epidemiológica de las enfermedades diarreicas agudas (EDA) en el Peru. Volume 27, SE-05. *Available at http://www.dge.gob.pe/portal/docs/vigilancia/boletines/2018/05.pdf*.

O'Ryan G, Ashkenazi-Hoffnung L, O'Ryan-Soriano M, Ashkenazi S. 2014. Management of acute infectious diarrhea for children living in resource-limited settings. *Expert Review of Anti-infective Therapy* **12**(**5**):621–632 DOI 10.1586/14787210.2014.901168.

Pekmez CT, Dragsted LO, Brahe LK. 2018. Gut microbiota alterations and dietary modulation in childhood malnutrition—the role of short chain fatty acids. *Clinical Nutrition* **30**:1–16.

Petrof EO, Gloor GB, Vanner SJ, Weese SJ. 2013. Stool substitute transplant therapy for the eradication of Clostridium difficile infection: 'RePOOPulating' the gut. *Microbiome* **1(1)**:3 DOI 10.1186/2049-2618-1-3.

Pieścik-Lech M, Shamir R, Guarino A, Szajewska H. 2013. Review article: the management of acute gastroenteritis in children. *Alimentary Pharmacology and Therapeutics* **37**:289–303 DOI 10.1111/apt.12163.

Pittayanon R, Lau JT, Leontiadis GI, Tse F, Yuan Y, Surette M, Moayyedi P. 2019. Differences in gut microbiota in patients with vs without inflammatory bowel diseases: a systematic review. *Gastroenterology* **158(4)**:930–946.e1.

Pushpanathan P, Mathew GS, Selvarajan S, Seshadri KG, Srikanth P. 2019. Gut microbiota and its mysteries. *Indian Journal of Medical Microbiology* **37(2)**:268–277 DOI 10.4103/ijmm.IJMM_19_373.

Ramakrishna B. 2013. Role of the gut microbiota in human nutrition and metabolism. *Journal of Gastroenterology and Hepatology* **28(4)**:9–17 DOI 10.1111/jgh.12294.

Rexroad J, Martin TT, McNeilly D, Godwin S, Middaugh CR. 2016. Thermal stability of adenovirus type 2 as a function of pH. *Journal of Pharmaceutical Sciences* **95(7)**:1469–1479.

Robles-Alonso VY, Guarner F. 2013. Progreso en el conocimiento de la microbiota intestinal humana. *Nutricion Hospitalaria* **28(3)**:553–557.

Schippa S, Pia-Conte M. 2014. Dysbiotic events in gut microbiota: impact on human health. *Nutrients* **6(1)**:5786–5805 DOI 10.3390/nu6125786.

Scott K, Antoine JM, Midtvedt T, Van Hemert S. 2015. Manipulating the gut microbiota to maintain health and treat disease. *Microbial Ecology in Health and Disease* **26**:258.

Shah M, Odoyo E, Wandera E, Kathiiko C, Bundi M, Miringu G, Guyo S, Komoto S, Nyangao J, Karama M, Tsuji T, Taniguchi K, Morita K, Ichinose Y. 2017. Burden of rotavirus and enteric bacterial pathogens among children under 5 years of age hospitalized with Diarrhea in Suburban and Rural Areas in Kenya. *Japanese Journal of Infectious Diseases* **70(4)**:442–447 DOI 10.7883/yoken.JJID.2016.398.

Shin NR, Whon TW, Bae JW. 2015. Proteobacteria: microbial signature of dysbiosis in gut microbiota. *Trends in Biotechnology* **33(9)**:496–503 DOI 10.1016/j.tibtech.2015.06.011.

Sommer F, Anderson JM, Bharti R, Raes J, Rosenstiel P. 2017. The resilience of the intestinal microbiota influences health and disease. *Nature Reviews. Microbiology* **15**:630–638 DOI 10.1038/nrmicro.2017.58.

Sun J, Kato I. 2016. Gut microbiota, inflammation and colorectal cancer. *Genes and Diseases* **3(2)**:130–143 DOI 10.1016/j.gendis.2016.03.004.

Szajewska H, Skórka A, Ruszczyński M, Gieruszczak-Białek D. 2013. Meta-analysis: lactobacillus GG for treating acute gastroenteritis in children–updated analysis of randomized controlled trials. *Alimentary Pharmacology and Therapeutics* **38(5)**:467–476 DOI 10.1111/apt.12403.

Thursby E, Juge N. 2017. Introduction to the human gut microbiota. *Biochemical Journal* **474(11)**:1823–1836 DOI 10.1042/BCJ20160510.

Tremaroli V, Bäckhed F. 2012. Functional interactions between the gut microbiota and host metabolism. *Nature* **489**:242–249 DOI 10.1038/nature11552.

Van den Elsen L, Poyntz H, Weyrich L, Young W, Forbes-Blom E. 2017. Embracing the gut microbiota: the new frontier for inflammatory and infectious diseases. *Clinical & Translational Immunology* **6(1)**:125–134 DOI 10.1038/cti.2016.91.

Wang X, Zhang L, Wang Y, Liu X, Zhang H, Liu Y, Shen N, Yang J, Gai Z. 2018. Gut microbiota dysbiosis is associated with Henoch-Schönlein Purpura in children. *International Immunopharmacology* **58**:1–8 DOI 10.1016/j.intimp.2018.03.003.

Wexler H. 2007. Bacteroides: the good, the bad, and the Nitty.Gritty. *Clinical Microbiology Reviews* **20(4)**:593–621 DOI 10.1128/CMR.00008-07.

Yu Z, Chen C, Kling D, Liu B, McCoy JM, Merighi M, Heidtman M, Newburg DS. 2013. The principal fucosylated oligosaccharides of human milk exhibit prebiotic properties on cultured infant microbiota. *Glycobiology* **23(2)**:169–177 DOI 10.1093/glycob/cws138.