Arsenic disturbs the gut microbiome of individuals in a disadvantaged community in Nepal

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ABSTRACT

Arsenic is ubiquitous in nature, highly toxic, and is particularly abundant in Southern Asia. While many studies have focused on areas like Bangladesh and West Bengal, India, disadvantaged regions within Nepal have also suffered from arsenic contamination levels, with wells and other water sources possessing arsenic contamination over the recommended WHO and EPA limit of 10 μg/L, some wells reporting levels as high as 500 μg/L. Despite the region's pronounced arsenic concentrations within community water sources, few investigations have been conducted to understand the impact of arsenic contamination on host gut microbiota health. This study aims to examine differential arsenic exposure on the gut microbiome structure within two disadvantaged communities in southern Nepal. Fecal samples (n = 42) were collected from members of the Mahuawa (n = 20) and Ghanashyampur (n = 22) communities in southern Nepal. The 16S rRNA gene was amplified from fecal samples using Illumina-tag PCR and subject to high-throughput sequencing to generate the bacterial community structure of each sample. Bioinformatics analysis and multivariate statistics were conducted to identify if specific fecal bacterial assemblages and predicted functions were correlated with urine arsenic concentration. Our results revealed unique assemblages of arsenic volatilizing and pathogenic bacteria positively correlated with increased arsenic concentration in individuals within the two respective communities. Additionally, we observed that commensal gut bacteria negatively correlated with increased arsenic concentration in the two respective communities. Our study has revealed that arsenic poses a broader human health risk than was previously known. It is influential in shaping the gut microbiome through its enrichment of arsenic volatilizing and pathogenic bacteria and subsequent depletion of gut commensals. This aspect of arsenic has the potential to debilitate healthy humans by contributing to disorders like heart and liver cancers and diabetes, and it has already been shown to contribute to serious diseases and disorders, including skin lesions, gangrene and several types of skin, renal, lung, and liver cancers in disadvantaged areas of the world like Nepal.

1. Introduction

In South East Asia, an event that many are calling “the worst mass poisoning in history” is underway [1]. Millions of people living along the Mekong, Yangtze, Red, and Ganges rivers are exposed to arsenic concentrations well over the WHO and EPA recommended limit of 10 μg/L (10 μg/L), with regions of India and Bangladesh reaching concentrations as high as 4730 μg/L [2, 3, 4, 9, 10, 28, 29, 30]. The high arsenic concentration can be mostly attributed to weathering of arsenic rich iron Fe (III) oxide sediments from the Himalayan Mountains, which has led to...
mass dispersal of arsenic rich sediments across South East Asia, much of it trapped in aquifer sands. Population growth in the past decade has led to increased demand for tube wells which expose trapped arsenic sulfides and pyrites to reductive dissolution and competitive replacement by stronger anions like phosphates and bicarbonates. While the arsenic crisis in Bangladesh and West Bengal, India has been well documented since the 1990s, it has only been in the last 18 years that countries like Vietnam and Cambodia have reported high arsenic concentration levels due to microbial contamination of surface water, installation of tubewell to prevent water borne diseases and reduce the children mortality [5].

Arsenic causes poisoning of the human body by disrupting glycolysis, namely by inhibiting acetyl-CoA and succinic dehydrogenase [6, 7]. Along with its metabolic disruption, arsenic exposure is known to increase the risk of hepatitis [8], several types of cancers, including lung and liver [9]), and has been linked to the development of type II diabetes mellitus [10, 11]. Arsenic’s influential role in the development of cancer is through binding to glutathione, a fundamental cellular antioxidant [12]. Once arsenic is bound, glutathione removes it from the cell. Higher cellular glutathione levels correspond with lower cellular arsenic levels. However, it has been reported that a low dietary intake of protein can lead to lower levels of intracellar glutathione in the liver [13, 14, 15]. Therefore, disadvantaged populations like those in South East Asia are particularly susceptible to the toxin.

In addition to the direct negative influence arsenic has on human health, its secondary effects through influence of the human gut microbiome are also well documented [16, 17, 18, 19]. Previous studies conducted by Dherr et al. [20] and Lu et al. [21] reported that adult mice exposed to arsenic display compositional and metabolic changes to their gastrointestinal tract microbiota. High concentrations of arsenic (10 μg/L) have also been shown to decrease levels bacteria commonly found within the human gut [20, 21, 22]. Arsenic can further alter the human gut microbiome by promoting the growth of arsenic-metabolizing bacteria like Bilophila, Desulfovibrio, and Bacillus [23, 24, 25, 26, 27]. These bacteria can metabolize arsenate (As(V)) and arsenite (As(III)), producing nitrogen metabolites toxic to the host [20].

While studies on the effects of arsenic on human health in Bangladesh and West Bengal, India in the past decade have been prolific, few studies have examined these same effects in Nepal and fewer have studied arsenic’s effect on the human gut microbiome. Our study represents one of the first to examine arsenic’s direct influence on the human gut microbiome. The goal of this study is to identify compositional changes to the gut microbiome induced by arsenic exposure that could be indicative of a pathogenic state. Further, this study will provide preliminary evidence to support supplementary studies that will examine the long-term effects of arsenic exposure on the microbiota in a disadvantaged population, such as that found in rural Nepal.

2. Methods

2.1. Sample collection

In this study, 44 low-income homes (Mahuawa (n = 20) and Ghanashyampur (n = 22)) in remote areas of Nepal, where arsenic is prevalent in potable well water were surveyed. This region of Nepal, Nawalparasi, has wells that report some of the highest levels of arsenic in the entire country, with some wells contaminated with as much as 500 μg/L arsenic [28]. This stands in contrast to wells in other districts of Nepal which generally report arsenic concentration levels that are below 10 μg/L [29]. Well arsenic concentrations are comparable to levels reported in Bangladesh of >200 μg/L, though falls short of one of the highest levels reported in that country of 4730 μg/L [28, 30]. The samples were collected from two different communities of Ghanashyampur and Mahuawa. Participants were recruited with the help of an NGO (non-governmental organization), the Environmental and Public Health Organization (ENPHO) based in Kathmandu, Nepal. Fecal and urine samples were collected from study participants for microbiome sequencing and arsenic testing, respectively. Volunteers were requested to provide answers to a screening questionnaire consisting of questions designed to gather basic information on the health of the residents, medical histories, the number and age, and the history and condition of the home. All protocols and procedures were IRB (Institutional Review Board) approved.

For this study, information on arsenic concentration in the urine, age, sex, and the community individuals were from as well as their VDC (Village Development Committee) was collected to inform the multivariate statistical analyses. For further analysis of the effects of arsenic on the gut microbiome, the arsenic concentration levels were categorically grouped by exposure level in the “Arsenic Exposure Level” metadata category. Individuals with urine arsenic concentrations of 0 μg/L were categorized as “Undetected”, those with urine arsenic concentrations between 0 μg/L and 10 μg/L were categorized as “Moderate” and individuals with urine arsenic concentrations above 10 μg/L were categorized as having a “High” exposure level. Urine samples were measured using Hybrid Generation/Atomic Absorption Spectrometry method following Standard Methods 5114B (APHA 2012) [69].

2.2. PCR amplification methods

Illumina iTag Polymerase Chain Reactions (PCR) were performed at a total volume of 25 μL for each sample and contained final concentrations of 1X PCR buffer, 0.8 mM dNTP’s, 0.2 μM 515F forward barcoded primer, 0.2 μM Illumina 806R reverse primer and ~10 ng of template DNA per reaction. PCR was carried out on a MJ Research PTC-200 thermocycler (Bio-Rad, Hercules, CA) using the following cycling conditions: 98°C for 3 min; 35 cycles of 98°C for 1 min, 55°C for 40 s, and 72°C for 1 min; 72°C for 10 min; and kept at 4°C. PCR products were visualized on a 1% SYBRsafe E-gel (ThermoFisher Scientific, Waltham, MA).

2.3. Library purification, verification and sequencing methods

Pooled PCR products were gel (2% Seakem LE agarose (Lonza Rockland, Inc, Rockland Maryland), stained with Gelstar (Lonza Rockland, Inc, Rockland Maryland) ethidium bromide) purified using the Qiagen Gel Purification Kit (Qiagen, Frederick, MD). Clean PCR products were quantified using the Qubit 2.0 Fluorometer (Life Technologies, Carlsbad, CA), and samples were combined in equimolar amounts. Prior to submission for sequencing, libraries were quality checked using the 2100 Bioanalyzer DNA 1000 chip (Agilent Technologies, Santa Clara, CA). Pooled libraries were stored at -20°C until they were shipped on dry ice to Laramar (Culver City, CA) for sequencing. Library pools were size verified using the Fragment Analyzer CE (Advanced Analytical Technologies Inc., Ames IA) and quantified using the Qubit High Sensitivity dsDNA kit (Life Technologies, Carlsbad, CA). After dilution to a final concentration of 1 nM and a 10% spike of PhiX V3 library (Illumina, San Diego CA), pools were denatured for 5 min in an equal volume of 0.1 N NaOH then further diluted to 12 pm in Illumina’s HT1 buffer. The denatured and PhiX-spiked 12 pm pool was loaded on an Illumina MiSeq V2 500 cycle kit cassette with 16S rRNA library sequencing primers and set for 250 base, paired-end reads. Arsenic levels in urine samples were measured using Atomic Absorption Spectroscopy at Environmental and Public Health Organization, Kathmandu, Nepal.

2.4. Quality filtering and OTU picking methods

Paired-end sequences were trimmed at a length of 251 bp and quality filtered at an expected error of less than 0.5% using USEARCH v7 [31]. After quality filtering, reads were analyzed using the QIIME 1.9.0 software package [32, 33]. Chimeric sequences were identified using USEARCH7 [34]. A total of 2,127,938 sequences were obtained after quality filtering and chimera checking. Open reference operational taxonomic units (OTUs) were picked using the USEARCH7 algorithm.
[34], and taxonomy assignment was performed using the Greengenes 16S rRNA gene database (13-5 release, 97%) [35]. Assigned taxonomy were organized into a BIOM formatted OTU (operational taxonomic unit) table, which was summarized at the genus level (L6) within QIIME 1.9.0 [32].

2.5. Microbial community analysis

Three separate OTU tables were used for analysis. The first contained all samples and was used to compare phylogenetic differences between communities. The second and third were filtered to contain only samples within the Ghanashyampur and Mahuwa communities, respectively. Principal coordinates analysis (PCoA) plots and ADONIS tests for significance were calculated from a weighted UniFrac distance matrix generated from a CSS normalized OTU table within Qiime 1.9.0 [32]. Distance box plots were generated using the weighted UniFrac distances to quantify the variation in sample composition observed in the PCoA plots. Adonis and ANOSIM tests for significance were calculated to determine significance of beta diversity clustering between experimental cohorts of interest grouped by arsenic concentration. ANOSIM and Adonis p-values under 0.05 were considered significant. Linear discriminant analysis effect size (LEfSe) biomarker identification analysis was conducted to identify significantly enriched (LDA >1, P < 0.05) bacterial taxa within each respective arsenic concentration cohort [36]. Genus-level relative abundances were multiplied by one million and were formatted as described in Segata et al. [36]. Initial comparisons were made with “Community” as the main categorical variable (Mahuwa or Ghanashyampur). Subsequent LEfSe comparisons were conducted with “Arsenic Exposure Level” (“Undetected”, “Moderate” and “High”) as the main categorical variable. Linear Discriminant Analysis scores for the enriched taxa within each class were then plotted. Features were plotted on a logarithmic scale according to the experimental group

| Sample ID | Community       | Arsenic Conc. in Urine | Arsenic Exposure Level | Age | Sex |
|-----------|-----------------|------------------------|------------------------|-----|-----|
| A1        | Ghanashyampur   | 0.026                  | High                   | 49  | M   |
| A2        | Ghanashyampur   | 0.026                  | High                   | 33  | M   |
| A3        | Ghanashyampur   | 0.014                  | High                   | 45  | F   |
| A5        | Ghanashyampur   | 0.045                  | High                   | 44  | F   |
| A6        | Ghanashyampur   | 0.012                  | High                   | 45  | F   |
| A7        | Ghanashyampur   | 0.011                  | High                   | 49  | M   |
| A8        | Ghanashyampur   | 0.013                  | High                   | 45  | M   |
| A9        | Ghanashyampur   | 0.009                  | Moderate               | 40  | F   |
| A10       | Ghanashyampur   | 0.011                  | High                   | 45  | F   |
| A11       | Ghanashyampur   | 0.013                  | High                   | 37  | F   |
| A13       | Ghanashyampur   | 0.019                  | High                   | 35  | F   |
| A14       | Ghanashyampur   | 0.027                  | High                   | 16  | F   |
| A15       | Ghanashyampur   | 0.014                  | High                   | 55  | F   |
| A16       | Ghanashyampur   | 0                      | Undetected             | 28  | M   |
| A17       | Ghanashyampur   | 0.012                  | High                   | 17  | F   |
| A18       | Ghanashyampur   | 0                      | Undetected             | 28  | M   |
| A19       | Ghanashyampur   | 0.103                  | High                   | 16  | M   |
| A20       | Ghanashyampur   | 0.021                  | High                   | 17  | F   |
| A21       | Ghanashyampur   | 0.008                  | Moderate               | 17  | M   |
| A22       | Ghanashyampur   | 0.013                  | High                   | 16  | F   |
| A24       | Ghanashyampur   | 0.009                  | Moderate               | 40  | F   |
| A25       | Ghanashyampur   | 0                      | Undetected             | 48  | F   |
| B1        | Mahuwa          | 0.011                  | High                   | 60  | M   |
| B2        | Mahuwa          | 0                      | Undetected             | 65  | M   |
| B3        | Mahuwa          | 0.006                  | Moderate               | 71  | F   |
| B4        | Mahuwa          | 0                      | Undetected             | 65  | F   |
| B5        | Mahuwa          | 0                      | Undetected             | 29  | M   |
| B6        | Mahuwa          | 0                      | Undetected             | 53  | F   |
| B7        | Mahuwa          | 0.008                  | Moderate               | 45  | F   |
| B8        | Mahuwa          | 0                      | Undetected             | 60  | F   |
| B9        | Mahuwa          | 0                      | Undetected             | 61  | M   |
| B10       | Mahuwa          | 0                      | Undetected             | 51  | F   |
| B11       | Mahuwa          | 0.013                  | High                   | 45  | F   |
| B12       | Mahuwa          | 0                      | Undetected             | 45  | F   |
| B13       | Mahuwa          | 0.008                  | Moderate               | 45  | M   |
| B14       | Mahuwa          | 0.005                  | Moderate               | 51  | F   |
| B16       | Mahuwa          | 0                      | Undetected             | 45  | M   |
| B17       | Mahuwa          | 0.006                  | Moderate               | 65  | M   |
| B18       | Mahuwa          | 0.05                   | High                   | 57  | M   |
| B19       | Mahuwa          | 0.035                  | High                   | 35  | F   |
| B20       | Mahuwa          | 0.031                  | High                   | 57  | M   |
| B23       | Mahuwa          | 0                      | Undetected             | 13  | F   |
to which they were significantly associated. LEfSe explicitly requires all pairwise comparisons to reject the null hypothesis for detecting the biomarker taxa, thus, no multiple testing corrections were needed [36].

Alpha diversity indices, including Chao1, Hei's evenness, Observed Species, and PD Whole Tree, were generated from an un rarified OTU table with a max sampling depth of 1600, a step size of 140, and with 20 iterations at each step. A CSS normalized OTU and an accompanying continuous metadata table were formatted for RStudio [37]. Correlation plots were generated in RStudio using the “Hmisc” [38] and “corrplot” [39]. Heatmaps were generated in RStudio using the “pheatmap” [40], “phyloseq” [41], “ggplot2” [42], “vegan” [43], “dplyr” [44], “scales” [45], “grid” [46], and “reshape2” [47] packages. Input OTU tables for each individual community were filtered so that only known pathogenic and arsenic volatilizing bacteria were visualized within the final correlation plot.

3. Results

3.1. Sequencing results and description of study population

A total of 44 fecal samples were collected from the Mahuwa (n = 20) and Ghanashyampur (n = 22) communities within Nepal for 16S RNA sequencing and analysis. The Illumina MiSeq yielded over 2,440,769 raw 16S rRNA sequences across all samples of which 2,127,938 sequences were retained after quality filtering and chimera checking. The mean arsenic concentration for the Mahuwa community (n = 20) was 0.009 (±0.014) μg/L and 0.018 (±0.021) μg/L for the Ghanashyampur community (n = 22). A student’s t-test (p = 0.085) revealed that the difference in arsenic concentration level was not significant. Table 1 shows a summary of the key characteristics of the samples within each community. In addition, Table 2 shows the Adonis R² value, the degree to which specific metadata items contribute to differences in bacterial community composition within samples. When comparing samples from both communities, “Arsenic Concentration in Urine” contributed the most to differences in bacterial community composition.

3.2. Bacterial community structure comparisons

No significant differences in alpha diversity distance boxplots were observed between sampled communities or summarized (“Undetected”, “Moderate”, “High”) arsenic concentration groupings when considering the observed species richness metric (Figure 1). No significant differences existed between the mean observed species of each categorical grouping (Mahuwa_Undetected 73.505 observed species; Mahuwa_Moderate 54.26 observed species; Mahuwa_High 85.92 observed species; Ghanashyampur_Undetected 67.13 observed species; Ghanashyampur_Moderate 47.92 observed species; Ghanashyampur_High 59.98 observed species) (Figure 1). Though there were no significant differences between arsenic exposure groupings, comparison of the two communities through

Table 2. Adonis R² value describes the degree to which specific metadata items contribute to differences in bacterial community composition within samples. When comparing samples from both communities, “Arsenic Concentration in Urine” contributed the most to differences in bacterial community composition.

|                      | R²          |
|----------------------|------------|
| Community            | 0.0245 (2.45%) |
| Arsenic Concentration in Urine | 0.04365 (4.36%) |
| Age                  | 0.03998 (3.91%) |
| Arsenic Concentration in Urine | Ghanashyampur | 0.0517 (5.17%) |
|                      | Mahuwa     | 0.1407 (14.07%) |
|                      | Age        | 0.08458 (8.46%) |
|                      | Ghanashyampur | 0.07093 (7.09%) |
4. Discussion

In this study, fecal samples obtained from members of two separate communities in southern Nepal that have been heavily affected by arsenic contaminated water, were used to better understand the influence of arsenic exposure on the human gut microbiome. High throughput sequencing of the 16S rRNA gene revealed significant bacterial community responses to elevated measures of arsenic within each respective community. We observed two distinctly defined taxonomic compositions between the two communities, shaped by geographic separation and differences in arsenic concentration. Our finding supports a previous study that identified similar spatial and concentration related differences in bacterial community response to a toxin [48]. Our study is the first of its kind to analyze changes to the gut microbiota of individuals in South East Asia, and lays the groundwork for future, more in-depth studies on the influence of arsenic on the health of the gut microbiome and overall human health.

Within the Ghanashyampur community, a selection for pathogenic bacteria was observed, including the Treponema, causal factor of syphilis [49]. While selection of this bacterial taxon has not yet been specifically linked to arsenic exposure, a study conducted in 1917, commented on the fact that after treatment with arsenic, a common syphilis remedy at that time, the Treponema became resistant to the arsenic compounds used to treat the disease [50]. More recently, Shivani et al. [51] reported the presence of arsenic resistant genes in a related bacterium within the Spirochaetaceae family. The Spirochetes class, which includes the causal agents of Lyme’s disease and syphilis, were also elevated within the Ghanashyampur community [49, 52, 53]. Further, the Bilophila, a member of the Desulfovibrionaceae family and candidate arsenic volatilizing bacterium yielded the strongest positive correlation with arsenic of all identified taxonomy within the Ghanashyampur community [54]. This specific genus is not very common in the gut microbiome but is known to be sulfate reducing [55], a possible contributor to colorectal carcinogenesis [56] and has been noted to have geno- and cytotoxic effects on gut epithelial cells [57]. Desulfovibrionaceae was also positively correlated with arsenic concentration in urine. This bacterial family possesses three-member genera including Desulfovibrio, Bilophila and Lawsonia. Desulfovibrio and Bilophila are arsenic volatilizing, or play some role in metabolizing and producing arsenic compounds in the gut [23, 54] and perform their arsenic reducing activity in the reductive environment of the human gut [58]. Lawsonia by comparison has no known arsenic volatilizing activity and has only been observed as pathogenic to animals [59, 60, 61]. Further, the genus Clostridium was positively correlated with arsenic concentration in the urine. This observation is congruent with Oremland and Stolz’s [23] observation of the Clostridium species OhlAS as an arsenic volatilizing bacterium.
Figure 3. Cladogram comparing differences in significantly enriched taxa between the two communities.

Figure 4. Comparison of differences in community composition between samples. A) Mahuawa and B) Ghanashyampur community.
A defined structure of degradative bacteria was observed in the Mahuawa community as well, though with a noted absence of the Treponema and the other Spirochetes, but the presence of the Citrobacter. The Citrobacter have been observed to be opportunistic even in immunocompetent patients [62], and it has additionally been linked to arsenic volatilization [23]. The arsenic volatilizing bacteria Desulfovibrio and Clostridium were also selected for in the Mahuawa community [23]. An additional bacterium playing a degradative role in the Mahuawa community was the Bacillales family, which includes the genera Bacillus that is both pathogenic [63] and arsenic volatilizing [23]. The Mahuawa community, more so than the Ghanashyampur, was observed to have a depletion of specific gut commensal bacteria, Ruminococcus and Clostridiaceae. Both taxa fall within the Firmicutes phylum and interact closely with host gut epithelial cells, are fundamental in maintaining a healthy gut microbe composition, and protect the host from opportunistic pathogens [64, 65]. In their 2015 study Dheer et al. [20] sought to observe structural and compositional changes to the gut microbiota of mice exposed to different arsenic concentrations (none, 10 μg/L and 250 μg/L) over the course of 10 weeks. They observed a reduction of Firmicutes, like Clostridiaceae and Ruminococcus and noted increased sporulation, a dormant state that bacteria enter when toxins are present or the surrounding environment becomes too inhospitable, over the course of the 10-week experiment.

Additional depletions of members of the less well documented bacterial order, Erysipelotrichiales, were observed in both communities. This finding is supported by similar results from Lu et al. [21] who observed significant decrease in the relative abundance of Erysipelotrichaceae, a member family of Erysipelotrichiales, in animals that had fed food containing high (10ppm) levels of arsenic. To our knowledge Lu et al. [21] and ourselves are the first to report original research that shows relative abundances of Erysipelotrichales decrease in response to arsenic exposure. There have been no definitive results on the role of Erysipelotrichales in the gut, but a 2015 review by Kaakoush [66] postulated that it may play a role in host lipid metabolism. Kaakoush [66] ultimately concluded that further research is necessary to determine this taxa’s biological role in the body. Additionally, further research is necessary to learn more about the effects of arsenic on Erysipelotrichaceae.

Within the Ghanashyampur community, differential bacterial taxa were found to correlate with increased arsenic in comparison to the Mahuawa community. However, the identified taxonomic rankings in the two communities were found to carry out similar functions. Both communities shared selection for some arsenic volatilizing taxa like Desulfovibrioaceae, but each was possessive of its own unique assemblage of pathogenic bacteria and both showed higher selection for certain arsenic volatilizing bacteria that the other did not. As noted earlier, the observation of site specific compositional differences in response to a contaminant is not unique to this study [48].

Our study has shown that there are identifiable pathogens and arsenic volatilizing bacteria in the guts of individuals from both Nepalese communities. It has further determined that the specific levels of those bacteria differ between the two communities. Different arsenic volatilizing bacteria are dominant in the Ghanashyampur community than are dominant in the Mahuawa community. Future studies will aim to better understand this difference, and there are several methods that will help to refine and explain this difference. First, it will be necessary to revise the metric for determining arsenic exposure in an individual as arsenic concentration in the urine can’t discern the full range of arsenic exposure someone has experienced over their lifetime. To that end, it would be better to use hair or fingernail samples, which retain the toxin much longer and would thus provide a better determination of arsenic exposure over the course of an individual’s lifetime [67]. We would then be able to more accurately identify which individuals would be grouped into the “Undetected”, “Moderate” and “High” categorical arsenic concentration groupings which would lead to better profiling of bacterial compositional shifts and enrichment of bacteria when arsenic is present. Metatranscriptomic analysis is also necessary to better identify enriched genes. Discerning the presence of pathogenic genes in a cohort will be important to better understand how arsenic is, in fact, affecting the overall health of these individuals.

4.1. Limitations

At first, this study was sampling at only one time point which limited analysis of the effects of arsenic on the gut microbiome over an extended period. Secondly, no household water and diet rice samples were collected in this study due to limited funding. Finally, one of the most important additions to future studies in this area is a stronger control group. While there were individuals who registered 0 μg/L urine arsenic concentration there was no way to know whether individuals

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**Figure 5.** Correlation plots analyzing bacterial correlations with arsenic concentration in urine. The size of the dot and the color indicate strength of the correlation. Dark blue indicates a strong positive correlation while dark red indicates a strong negative correlation. (A) Correlations between arsenic concentration in urine and bacterial taxa within the Mahuawa community. Plotted correlations all have a p-value less than 0.05. (B) Correlations between arsenic concentration and bacterial taxa within the Ghanashyampur community. Only the most important additions to future studies in this area are noted.
classified in the “Undetected” arsenic concentration grouping, had never been exposed to the toxin, though the data suggest that they had all been exposed on some level due to the presence of indicator taxa and predicted gene families. The best control group would be from Nepal, but from an area that had better water quality control. Similar studies have used American samples [68], as there is more surety of limited to no arsenic contamination, but there are so many other confounding factors such as diet and influence of other environmental factors which makes the use of American samples as controls, highly biased. The hope is to better understand the role of arsenic in pathogenicity and gut dysbiosis, and how to remedy its effects.

Declarations

Author contribution statement

Jeffrey L Brabec: Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Justin Wright: Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Truc Ly, Vasily Tokarev: Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

Hoi Tong Wong, Christopher J McClimans: Analyzed and interpreted the data.

Regina Lamendella: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; wrote the paper.

Shardulendra Sherchand, Dipendra Shrestha, Sital Uprety, Bipin Dangol, Sarmila Tandukar, Jeevan B. Sherchand: Performed the experiments.

Samendra P Sherchan: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; wrote the paper.

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Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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