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Gut Microbiome Perturbations Induced by Bacterial Infection Affect Arsenic Biotransformation

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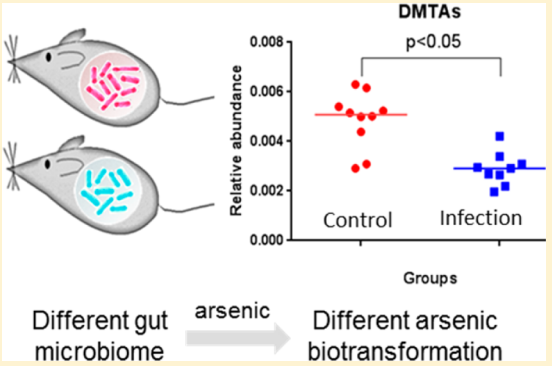
Supporting Information

ABSTRACT: Exposure to arsenic affects large human populations worldwide and has been associated with a long list of human diseases, including skin, bladder, lung, and liver cancers, diabetes, and cardiovascular disorders. In addition, there are large individual differences in susceptibility to arsenic-induced diseases, which are frequently associated with different patterns of arsenic metabolism. Several underlying mechanisms, such as genetic polymorphisms and epigenetics, have been proposed, as these factors closely impact the individuals' capacity to metabolize arsenic. In this context, the role of the gut microbiome in directly metabolizing arsenic and triggering systemic responses in diverse organs raises the possibility that perturbations of the gut microbial communities affect the spectrum of metabolized arsenic species and subsequent toxicological effects. In this study, we used an animal model with an altered gut microbiome induced by bacterial infection, 16S rRNA gene sequencing, and inductively coupled plasma mass spectrometry-based arsenic speciation to examine the effect of gut microbiome perturbations on the biotransformation of arsenic. Metagenomics sequencing revealed that bacterial infection significantly perturbed the gut microbiome composition in C57BL/6 mice, which in turn resulted in altered spectra of arsenic metabolites in urine, with inorganic arsenic species and methylated and thiolated arsenic being perturbed. These data clearly illustrated that gut microbiome phenotypes significantly affected arsenic metabolic reactions, including reduction, methylation, and thiolation. These findings improve our understanding of how infectious diseases and environmental exposure interact and may also provide novel insight regarding the gut microbiome composition as a new risk factor of individual susceptibility to environmental chemicals.



1. INTRODUCTION

Exposure to arsenic affects large human populations worldwide through the contamination of drinking water by geological sources of inorganic arsenic. Hundreds of millions of people around the world, especially in South and East Asia, drink water with inorganic arsenic levels that far exceed the 10 μg/L guideline, established or accepted by the World Health Organization and the U.S. Environmental Protection Agency (EPA).[1] In the United States, as many as 25 million people are estimated to drink water with an arsenic level of >10 μg/L, as private wells are not regulated by the EPA or other agencies.[2] Arsenic exposure has been associated with a number of diseases, such as skin, bladder, lung, and liver cancers, diabetes, and cardiovascular disorders and reproductive defects.[1,3−5] More recently, arsenic exposure has been linked to an increased incidence of diabetes in animal models and human population studies.[6,7] Numerous mechanisms, including interaction with sulfur, oxidative stress, genotoxicity, altered DNA repair and signal transduction, cell proliferation, and epigenetics, have been proposed for arsenic-induced diseases.[1,3,8−10] In addition, there are large differences in susceptibility to arsenic-induced diseases among individuals,[11,12] with several underlying mechanisms, such as genetic polymorphisms, epigenetics, and nutrition homeostasis, being proposed. Individual susceptibility is frequently associated with different spectra of arsenic metabolism. Accumulating evidence indicates that perturbations of the gut microbiome and functions may play an important role in the development of human diseases. The essential role of the gut microbiome in a variety of aspects of human health and metabolic processing of xenobiotics raises the possibility that gut microbiome phenotypes affect the

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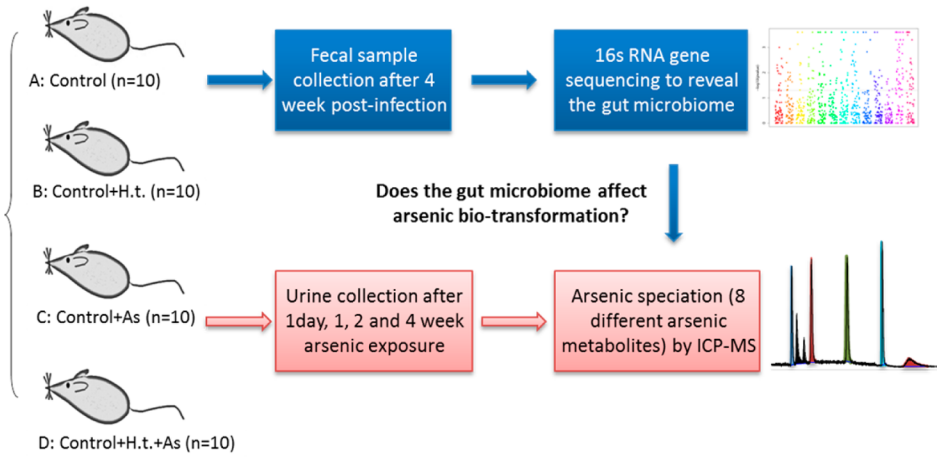

Figure 1. Integration approach combining a bacterial infection animal model, 16S rRNA gene sequencing, and ICP-MS-based arsenic speciation for exploring the impact of gut microbiome changes on the biotransformation of arsenic (*H. trogunturn*, H.t.).

biotransformation of arsenic. This hypothesis is supported by several previous studies that have clearly demonstrated the involvement of the gut microbiome in the reduction, methylation, and thiolation of arsenic species.[13,14]

The human body harbors 100 trillion gut microbes, ~10 times more than all human cells.[15] It is estimated that the ~500−1000 species residing in the human gut encode 100-fold more unique genes than our human genome. The gut microbiota has important functions in metabolic processing, energy production, immune cell development, food digestion, epithelial homeostasis, etc.[16,17] Mounting evidence indicates that dysregulated gut microflora contributes to a variety of diseases, including diabetes, obesity, cardiovascular diseases, allergies, inflammatory bowel disease, and others.[18−21] The composition of the gut microbiome is highly diverse, and this diversity can be readily affected by external factors, such as environment, diet, bacterial/viral infection, and antibiotics. Furthermore, previous studies have shown that altered gut microflora dramatically changed activities of diverse enzymes in the liver, including P450s and phase II enzymes responsible for the metabolic activation of xenobiotics.[22] This raises the possibility that gut microbiome phenotypes play a role in defining the individual response by altering the metabolic capacity in the host upon exposure to environmental chemicals.

In particular, inorganic arsenic is metabolized via a series of methylation and reduction reactions, with *S*-adenosylmethionine (SAM) as the main methyl donor.[23,24] The widely accepted metabolic pathway of inorganic arsenic consists of alternating reduction from pentavalent arsenic to trivalent and oxidative methylation of the trivalent arsenic metabolites. The six arsenic species involved in this pathway in humans are inorganic arsenic (iAsV and iAsIII), monomethylarsonic acid (MMAsV), monomethylarsonous acid (MMAsIII), dimethylarsinic acid (DMAsV), and dimethylarsinous acid (DMAsIII). In addition to arsenic metabolism catalyzed by specific enzymes, such as arsenic (+3 oxidation state) methyltransferase (As3mt), in diverse tissues,[25] a few experiments have demonstrated that the gut microbiome participates in the metabolism of arsenic species.[13,14,26,27] For example, Rowland and Davies reported the reduction of iAsV to iAsIII, as well as the formation of MMAs and DMAs, by rat cecal bacteria.[28] Likewise, the thiolation of methylated As oxides in the cecal contents of a mouse[13] and the *in vivo* thiolation of arsenic have been reported.[29−31] More recently, Van de Wiele et al. found a high degree of methylation of iAs- and As-contaminated soils by human gut microbiota.[14] Besides the formation of MMAsV, they also detected the formation of highly toxic MMAsIII, and microbial thiolated monomethylmonothioarsonic acid (MMMTAsV).[14]

Given the role of the gut microbiome in direct metabolic reactions of arsenic and triggered systemic responses in other organs, we thus hypothesize that perturbations of the gut microbial community affect the spectrum of metabolized arsenic species and subsequent toxicological responses. In this study, we used an animal model with an altered gut microbiome, 16S rRNA gene sequencing, and inductively coupled plasma mass spectrometry (ICP-MS)-based arsenic speciation to examine the effect of gut microbiome perturbations on the biotransformation of arsenic. Metagenomics sequencing revealed that bacterial infection significantly perturbed the gut microbiome composition in C57BL/6 mice, which in turn resulted in altered spectra of arsenic metabolites, with inorganic arsenic species and methylated arsenic being upregulated and downregulated, respectively. These data clearly illustrated that gut microbiome perturbations arising from bacterial infection significantly affect the metabolism of arsenic. These findings may provide novel insight regarding the gut microbiome composition as a new risk factor of individual susceptibility to environmental chemicals.

2. MATERIALS AND METHODS

2.1. Chemicals. Sodium arsenite and arsenobetaine (AsB) were obtained from Fisher Scientific (Pittsburgh, PA) and Sigma-Aldrich (Milwaukee, WI), respectively. Dimethylarsinic acid (DMAsV) and disodium monomethylarsonate (MMAsV) were obtained from Chem Service (West Chester, PA). MMAsIII (oxomethylarsine), DMAsIII (iododimethylarsine), dimethylmonothioarsinic acid (DMTAs), and trimethyl arsine oxide (TMAsO) were provided by W. Cullen (University of British Columbia, Vancouver, BC). Other reagents used for the HPLC mobile phase and diluent were Puratonic 99.999% purity grade ammonium carbonate (Alfa Aesar, Ward Hill, MA), ammonium sulfate (Mallinckrodt, Hazelwood, MO), and ammonium acetate (ICN Biochemicals, Aurora, OH).

2.2. Animal Infection and Exposure. *Helicobacter*-free C57BL/6 (~8-week-old) mice were purchased from Jackson Laboratories (Bar Harbor, ME). Mice were provided pelleted rodent diet (ProLab 3000, Purina Mills, St. Louis, MO) and filtered water ad libitum and were maintained in AAALAC-accredited facilities in microisolator caging under standard environmental conditions. All experiments were

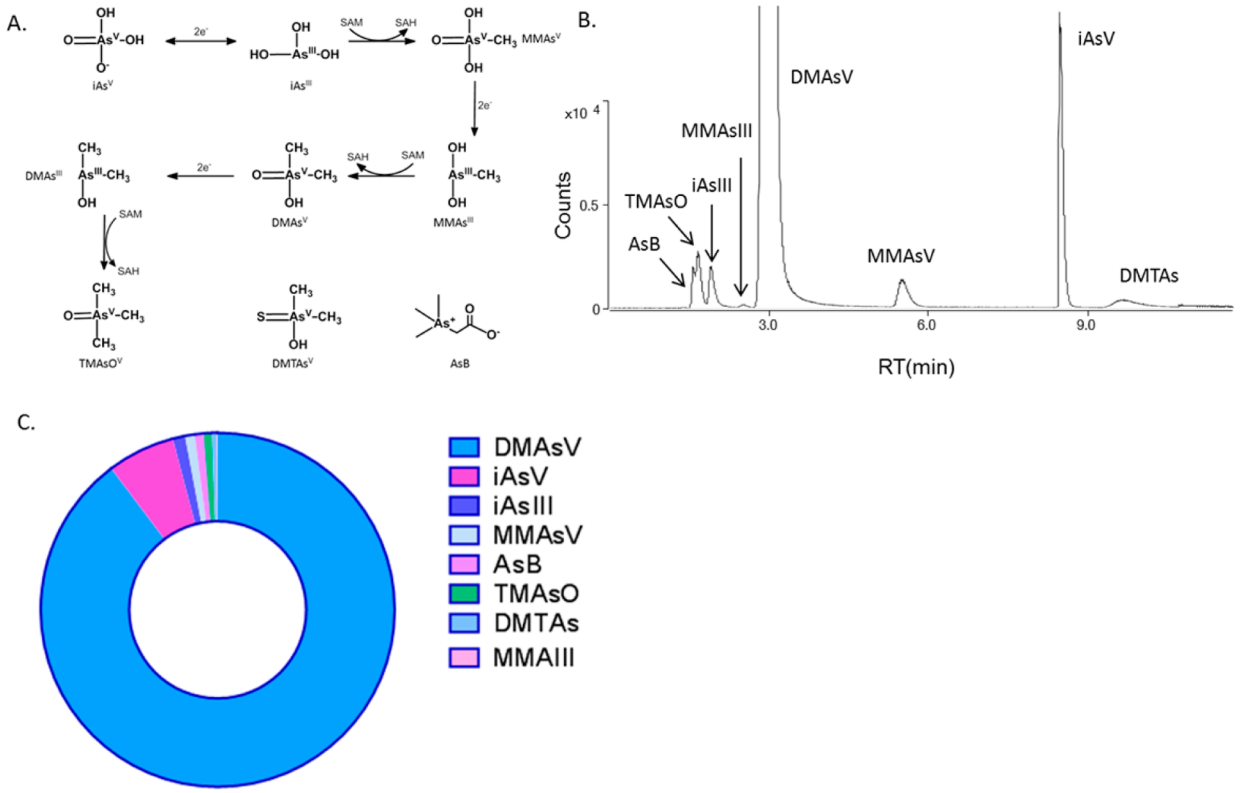

Figure 2. (A) Metabolism pathway and major arsenic species, (B) ICP-MS chromatogram of a urine sample from a mouse exposed to 10 ppm arsenic, and (C) relative abundance of different arsenic species after normalization using the total counts of arsenic at m/z 75, with DMAsV and iAsV being the top two arsenic metabolites.

approved by the Massachusetts Institute of Technology Committee on Animal Care. Each grouping comprised 10 mice unless stated otherwise. Mice (groups B and C only, Figure 1) were dosed at 9–10 weeks of age with 2×10^7 *Helicobacter trogontum* three times on alternate days by oral gavage. Inorganic arsenic (10 ppm) was then administered to mice (groups B and D only, Figure 1) through drinking water for 4 weeks. The *Helicobacter* infection status of these separately housed colonies was confirmed by fecal PCR at the end of the study. Urine samples were collected using a metabolic cage with dry ice placed around the urine collection vessel to prevent oxidation or degradation of metabolites during the collection period (~16 h). Fecal pellets were also collected from individual animals. Plasma samples and tissues were collected during necropsy at the end of the study.

2.3. Animal Monitoring and Histological Analysis. Throughout the experiments, mice were assessed for evidence of diarrhea, dehydration, and deteriorating body condition. Mice were euthanized with CO_2 and necropsied after 4 weeks of arsenic consumption. Formalin-fixed tissues were routinely processed, embedded in paraffin, sectioned at 4 μm, stained with hematoxylin and eosin, and evaluated by a board-certified veterinary pathologist blinded to the identity of the sample. Inflammation, edema, epithelial defects, hyperplasia, and dysplasia of multiple regions of liver and colon were scored on an ascending scale (0 to 4, with 0 being normal) of severity and invasiveness of the lesion if any.

2.4. 16S rRNA Metagenomics Sequencing. DNA was isolated from fecal pellets using a PowerSoil DNA Isolation Kit as instructed by the manufacturer (MO BIO Laboratories). The resultant DNA was quantified by UV spectroscopy and stored at −80 °C for further analysis. DNA was amplified using universal primers of U515 (GTGCCAGCMGCCGCGGTAA) and E786 (GGACTACHVGGGTWTCTAAT) to target the V4 regions of 16S rRNA of bacteria. Individual samples were barcoded and pooled to construct the sequencing library, followed by sequencing with an Illumina Miseq instrument to generate pair-ended 150 × 150 reads.

2.5. Data Analysis of 16s rRNA Sequencing Data. The raw mate-paired fastq files were quality-filtered, demultiplexed, and analyzed using quantitative insights into microbial ecology (QIIME).[32] For quality filtering, the default parameters of QIIME were maintained in which reads with a minimal Phred quality score of <20, containing ambiguous base calls and containing fewer than 113 bp of consecutive high-quality base calls, were discarded. Additionally, reads with three consecutive low-quality bases were truncated. The samples sequenced were demultiplexed using 8 bp barcodes, allowing 1.5 errors in the barcode. UCLUST was used to choose the operational taxonomic units (OTUs) with a threshold of 97% sequence similarity.[33] A representative set of sequences from each OTU were selected for taxonomic identification of each OTU using the Ribosomal Database Project (RDP) classifier.[34] The Greengenes OTUs (4feb2011 build) reference sequences (97% similar) were used as the training sequences for RDP. A confidence threshold of 0.80 was used for taxonomic assignment.

2.6. Arsenic Speciation. Arsenic species were measured using an Agilent (Santa Clara, CA) 7500 ICP-MS instrument, as described previously.[35] The ICP-MS instrument was interfaced with an Agilent 1260 HPLC system, and a Hamilton PRP-X100 column was used to separate arsenic species. Mobile phase A consisted of 10 mM ammonium carbonate and 10 mM TRIS (pH 8.7), and mobile phase B included 10 mM ammonium carbonate, 10 mM TRIS, and 15 mM ammonium sulfate (pH 8.0). The following gradient was run: 0 to 100% B from 0 to 5 min, 100% B from 5 to 11 min, and 100% A from 11 to 16 min. Sample preparation was conducted as follows. Ten microliters of a urine sample or arsenic reference standards was diluted with 0.1 M ammonium acetate (pH 5) to yield a total volume of 40 μL in a polypropylene microcentrifuge tube. Microcentrifuge tubes containing samples were centrifuged for 5 min at 12000 rpm and 4 °C in a refrigerated centrifuge. Following centrifugation, 35 μL of the supernatant was transferred to an HPLC autosampler vial, without disturbing the solid pellet at the bottom of the tube, followed by injection of 5–10 μL into the mass spectrometer.

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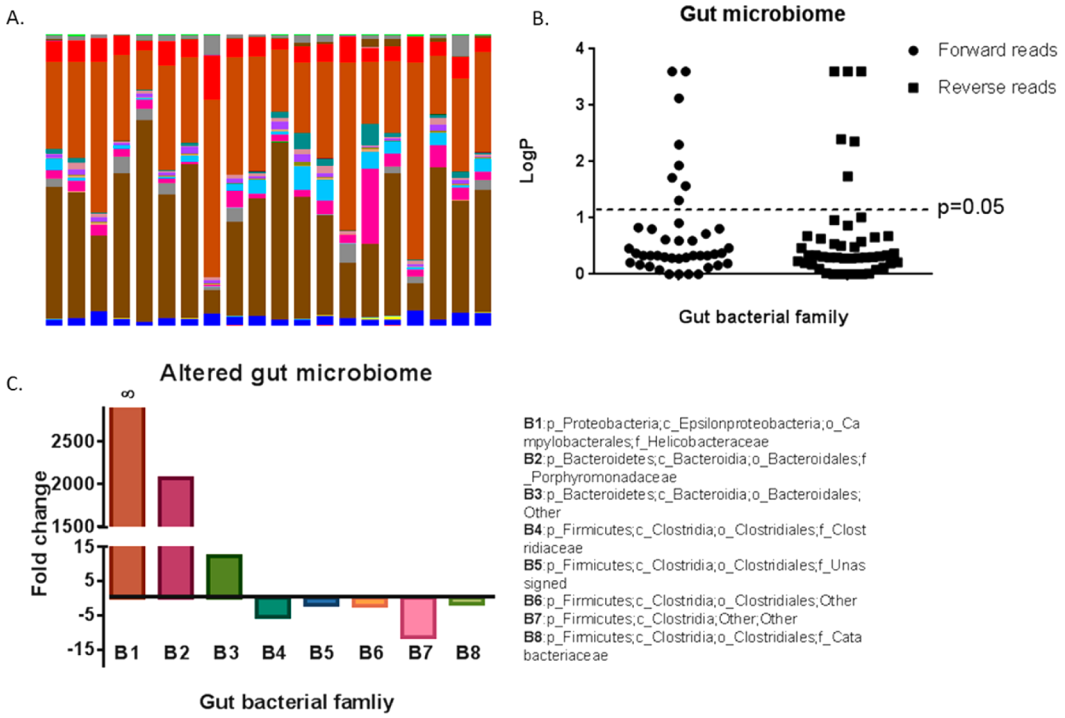

Figure 3. (A) Major gut bacteria at the family level in individual animals, as revealed by 16S rRNA sequencing, with each color being one bacterial family. (B) Statistically significantly altered gut bacterial families [those above the dotted line ($p < 0.05$)] by 16S rRNA gene sequencing forward and reverse reads. (C) Fold changes and taxonomic assignments of perturbed gut bacteria at the family level in infected mice compared to controls. Abbreviations: p, phylum; c, class; o, order; f, family; ∞, *Helicobacter* detected only in the infection group.

2.7. Statistical Analysis. Data analysis was conducted with multivariate statistical methods to compare gut microbiome communities between groups. Principal coordinate analysis (PCoA) was performed to examine intrinsic clusters within the observations. In addition, jackknifed β diversity and hierarchical clustering analysis via unweighted pair group method with the arithmetic mean (UPGMAD) was used to differentiate the gut microbiome profiles of the controls and infected samples. The difference in the individual gut microbiome composition between control and treatment groups was assessed using a nonparametric test with Metastats as described previously.[36] The abundances of arsenic species in the control and treatment groups were compared with a Student's t test, and results were considered significant when $p < 0.05$. The correlation between different arsenic metabolites was generated using Pearson's correlation coefficient. One-way ANOVA with repeated measures was used to assess the effect of the time of gut microbiome changes on arsenic metabolites. A linear mixed statistical model was also applied to examine the impact of gut microbiome alterations on the formation of arsenic species. All data handling and statistical analyses were performed using the statistical R package or SAS.

3. RESULTS

3.1. Experimental Workflow. Figure 1 shows the experimental workflow for 16S rRNA sequencing and arsenic metabolite profiling. First, two groups of mice (groups B and D, 10 per group) were infected with *H. trogontum* with three doses in a week, as described previously.[37] Next, arsenic was administrated to two groups of mice (one control and another consisting of mice with altered gut microbiome arising from bacterial infection, i.e., groups C and D in Figure 1). For gut microbiome profiling, DNA was isolated from fecal pellets, amplified by PCR using 16S rRNA specific primers, and followed by 150 × 150 bp paired-end sequencing using an Illumina Miseq platform. The resultant sequencing reads were processed by the QIIME and Metastats software packages to reveal infection-induced gut microbiome changes. For arsenic speciation analysis, urine was collected and analyzed by HPLC–ICP-MS using a Hamilton PRP-X100 column. The resultant intensity counts (m/z 75) of each arsenic metabolite were further normalized to reduce the impact of individual variations of animals on the uptake of arsenic during exposure, followed by statistical analysis to reveal any altered metabolites between the control and treatment group. Urine samples were collected at multiple time points, including 1 day, 1 week, 2 weeks, and 4 weeks, to examine potential dynamic effects of gut microbiome changes on the metabolism of arsenic.

3.2. Arsenic Speciation by ICP-MS. Figure 2A illustrates an arsenic metabolism pathway that involves reduction, methylation, and thiolation, with eight major arsenic metabolites being measured. The formation of DMTAsV is unique, as gut bacteria are important in the generation of this thiolated arsenic metabolite. Figure 2B shows a HPLC–ICP-MS chromatogram from the urine sample of a mouse exposed to iAsIII for 1 week. AsB and TMAsO eluted first but overlapped, followed by iAsIII, MMAsIII, DMAsV, MMAsV, iAsV, and DMTAsV. Among these arsenic species, DMAsV is predominant (~90% of the counts), followed by iAsV (~5% of the counts), and the remaining arsenic metabolites account for a total of ~5% of the counts, as illustrated in Figure 2C. DMAsIII could not be resolved on the column as it coeluted with the large peak of DMAsV.

3.3. Gut Microbiome Changes Induced by Bacteria. As described in Materials and Methods, *H. trogontum* was used to disturb the gut microbiome to construct an animal model with bacterially induced gut microbiome alterations and also to serve as a model for understanding how infection influences an individual's response to environmental chemicals.

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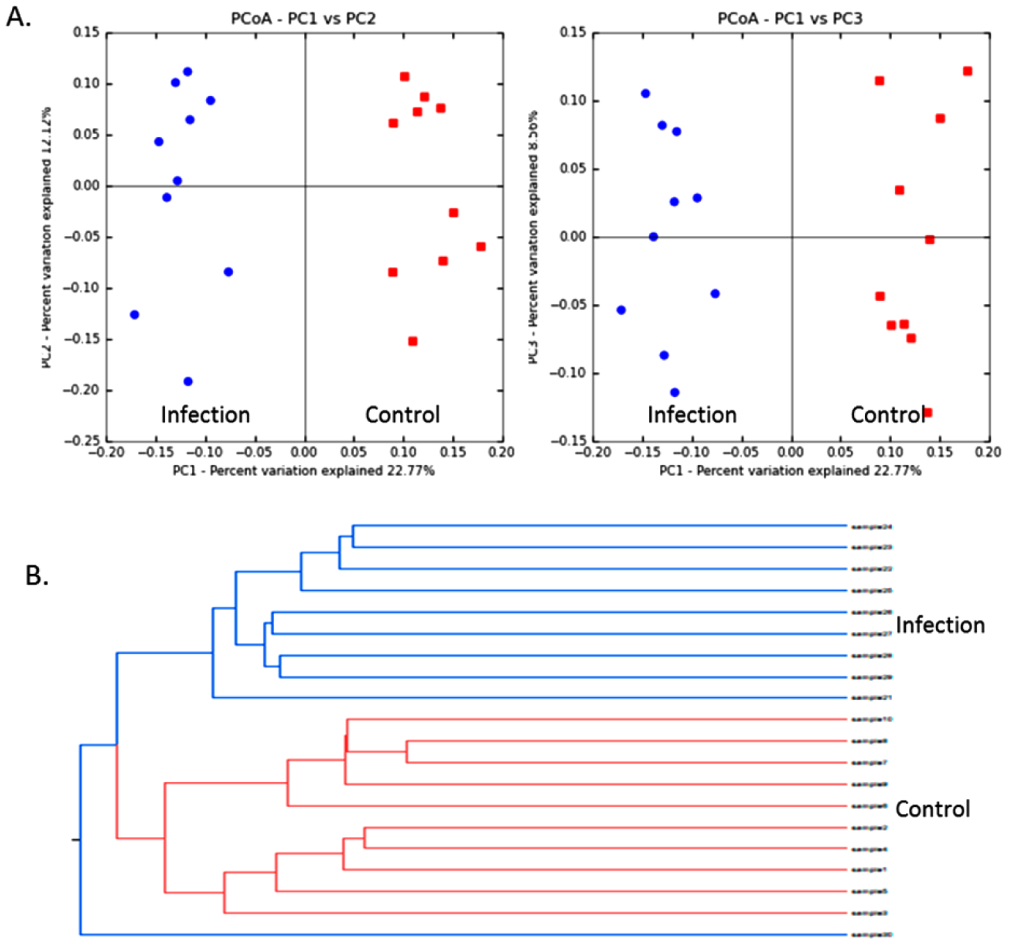

Figure 4. (A) Gut microbiome patterns of control samples (red) and infected (blue) mice are readily differentiated by PCoA, with 22.8, 12.1, and 8.6% variation explained by PC1−PC3, respectively. (B) The hierarchical clustering analysis by unweighted pair group method with the arithmetic mean indicates that controls and infected mice cluster in their own groups (red for control and blue for the treatment group).

To probe gut microbiome changes arising from bacterial infection, 16S rRNA gene sequencing was conducted. Figure 3A shows the identified gut bacteria assigned at the family level from 16S rRNA sequencing reads, with each color representing an individual bacterial family. In terms of the assignment at the phylum level, Fimicutes (53.7%) and Bacteroidetes (41.1%) are predominant in the gut bacteria of mice, followed by Tenericutes (2.7%), Actinobacteria (0.2%), Cyanobacteria (0.05%), and Proteobacteria (0.02%), with 2.2% of the sequences unmatched with the database (Figure S1 of the Supporting Information). The observations and assignments of gut bacteria at the phylum level are consistent with previous reports that the gut microbiome consists of only several phyla.[38] Figure 3B further illustrates all the gut bacteria families in control and infection groups, as revealed by 16S sequencing at the family level. The forward read and reverse read identify similar numbers and consistent types of gut bacteria at the family level, with 42 and 46 for the forward and reverse read, respectively. Significantly changed families of gut bacteria [those above the dotted line ($p < 0.05$)] are illustrated in Figure 3B, and their family level taxonomic assignments and fold changes are illustrated in Figure 3C. In addition to *Helicobacter* detected in the infection group only, two Bacteroidete families increased ~2000- and 15-fold compared to control (p_Bacteroidetes;c_Bacteroidia;o_Bacteroidales;f_Porphyromonadaceae and p_Bacteroidetes;c_Bacteroidia;o_Bacteroidales;Other, respectively). Five bacterial families belonging to Firmicutes were all downregulated in infected animals, with changes from −2- to −11-fold.

The difference in the gut microbiome patterns arising from *H. trogontum* infection could be readily differentiated upon comparison of β diversity UniFrac distances,[39,40] as shown by the PCoA plot in Figure 4A. The control (red) and treated (blue) animals are well separated, with 22.8, 12.1, and 8.6% variation explained by PC1−PC3, respectively. Consistent with the PCoA plot, the jackknifed β diversity and hierarchical clustering analysis via UPGMAD demonstrates that infected animals and controls typically cluster in their own groups, with one treated mouse being grouped with the control group, as shown in Figure 4B.

3.4. Histological Analysis. Histological analysis was also conducted to examine whether *H. trogontum* infection caused detectable tissue pathological changes. Histological scoring revealed no statistically significant differences between the controls and infected animals in terms of inflammation, edema, epithelial defects, crypt atrophy, hyperplasia, and dysplasia in multiple regions of the liver and colon (Figure S2 of the Supporting Information). These histological results are consistent with previous studies, which reveal that C57BL/6 mice are not susceptible to lower-bowel inflammation due to *Helicobacter* spp. infection, despite persistent infection.[37]

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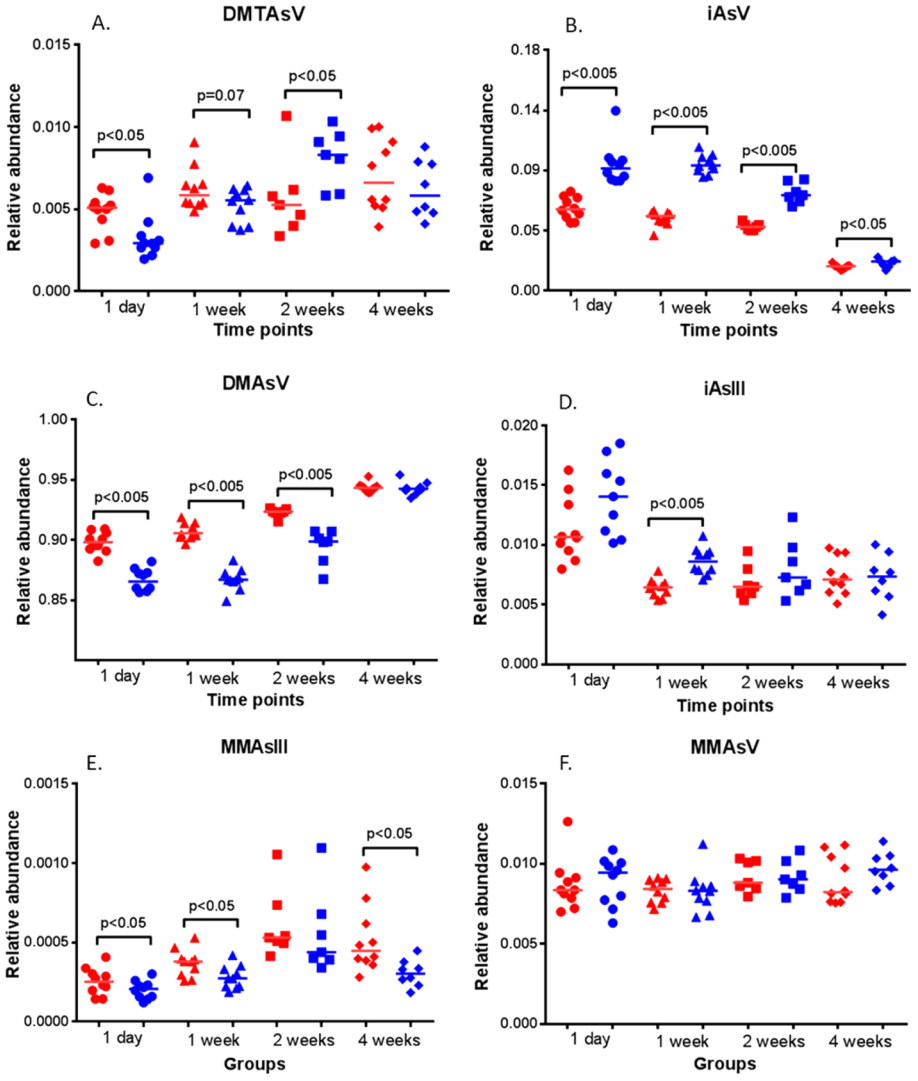

Figure 5. Arsenic metabolites were statistically significantly disturbed in infected animals compared to the controls ($p < 0.05$), with inorganic arsenic species and methylated and thiolated arsenic metabolites being perturbed at multiple time points: DMTAsV (A), iAsV (B), DMAsV (C), iAsIII (D), MMAsIII (E), and MMAsV (F) (red for controls and blue for infection groups).

3.5. The Gut Microbiome Affects the Metabolism of Arsenic. As shown in Figures 3 and 4, the gut microbiome was significantly perturbed by *H. trogontum* infection. We next compared the relative abundance of measured arsenic species to examine whether the gut microbiome alterations impact the biotransformation of arsenic, as shown in Figure 5A–F. Levels of DMTAsV, a gut-flora-generated thiolated arsenic metabolite, DMAsV, and MMAsIII were significantly decreased after arsenic exposure for 1 day, while the level of iAsV was increased in the infection group, suggesting that gut microbiome changes resulting from bacterial infection inhibit the detoxification of arsenic. Moreover, inorganic arsenic (iAsV) in urine from mice exposed for 1 day is highly negatively correlated with methylated and thiolated arsenic metabolites, including DMAsV and DMTAsV, as shown in Figure S3 of the Supporting Information. Similar to the case of exposure for 1 day, the level of iAsV was increased, while methylated arsenic metabolites, such as DMAsV, were significantly downregulated at other time points, including 1 week or 2 weeks post-arsenic exposure. These findings clearly indicate that bacterial infection-induced gut microbiome changes alter the reduction, methylation, and thiolation of arsenic and consequently inhibit its detoxification.

It has been well documented that the establishment or recovery of the gut microbiome is a temporal process.[41−43] Thus, the interaction between the gut microbiome and arsenic metabolism could be dynamic. Panels A and B of Figure S4 of the Supporting Information illustrate the effects of the time of gut microbiome on the formation of DMTAsV. Although there is no time dependence for DMTAsV in the control groups, a statistically significant time-dependent effect was observed in infection groups ($p < 0.005$), highlighting a potential dynamic impact of gut microbiome changes on the biotransformation of arsenic. Time-dependent effects were observed for the formation of DMAsV in both controls and treated animals, as shown in panels C and D of Figure S4 of the Supporting Information. However, a linear mixed statistical model further identified the influence of gut microbiome perturbations on the methylation of arsenic, with intercepts of 0.0017 and 0.0032 being determined for the control and treatment groups, respectively.

4. DISCUSSION

In this study, we used a bacterial infection animal model, high-throughput 16S rRNA gene sequencing, and ICP-MS-based arsenic speciation to study the impact of bacterially induced gut microbiome perturbations on the biotransformation of arsenic. The data clearly demonstrated that bacterial infection caused significant changes in the gut microbiome of mice, which in turn affected the spectrum of metabolized arsenic species in mice. These findings may provide novel mechanistic insights regarding the gut microbiome as a new risk factor of individual responses upon exposure to diverse environmental chemicals.

We used bacterially infected mice as an animal model to probe the impact of gut microbiome changes on arsenic metabolism. This selection was grounded in the understanding that bacterial infections are naturally occurring and universal. More importantly, infectious diseases have been intertwined with environmental exposure in complex human exposure scenarios, but the role of infection is not often well-considered in the common toxicological paradigm.[44] Our results are early insights into the relationships between bacterial infections and environmental exposures, and their consequent impacts on the toxicity of toxicants. Specifically, we discovered that several families of gut bacteria changed arising from *H. trogontum* infection, as shown in Figure 3. In addition to the *H. trogontum*, two other Bacteroidete families were significantly upregulated. Their levels increased ∼2000- and 15-fold compared to the control (p_Bacteroidetes;c_Bacteroidia;o_Bacteroidales;f_Porphyromonadaceae and p_Bacteroidetes;c_Bacteroidia;o_Bacteroidales;Other, respectively). Porphyromonadaceae is a family of pathogenic bacteria generally composed of two genera of environmental bacteria, *g_Porphyromonas* and *g_Dysgonomonas*.[45] Thus, it appears that *H. trogontum* infection increases the rate of expansion of opportunistic pathogens. Five bacterial families belonging to Firmicutes were all downregulated in infected animals, with changes ranging from −2- to −11-fold. These data clearly demonstrated that bacterial infection significantly altered the gut microbiome. It should be noted that our analyses were conducted at the family level because of the higher degree of confidence in the assignment of taxa based on the sequencing reads; therefore, a significant change at the family level may reflect changes of multiple gut bacteria at genus and species levels.

To examine whether infection and perturbed gut microbiome impact the biotransformation of arsenic, we measured arsenic species in urine and found a significant change in the spectrum of arsenic metabolites. Among these disturbed arsenic metabolites, DMTAsV is of particular interest, because previous studies have documented that thiolation of arsenic could be conducted by the gut microflora.[13,14] For example, the capacity of the anaerobic microflora from a mouse cecum to metabolize dimethylarsinic acid was examined, and DMAsV was thiolated to DMTAsV and dimethyldithioarsinic acid (DMDTAs) after incubation under anaerobic conditions at 37 °C for up to 24 h.[13] Another study, taking advantage of a dynamic model of the human gut, showed that microbial thiolation led to an additional thiolated arsenic metabolite, MMMTAsV, after incubation of the colon microbiata with iAs- and As-contaminated urban soils.[14] Moreover, urine analysis after iAs exposure has already revealed the formation of both DMTAsV and MMMTAsV in animals and humans.[46,47] In this study, we measured the abundance of DMTAsV, which was significantly different between the control and treatment group 1 day and 2 week post-arsenic exposure. For example, the level of DMTAsV was decreased in urine (approximately −1.5-fold change; $p < 0.05$) after a 1 day exposure in mice with a disturbed gut microbiome, highlighting the role of the gut flora in the generation of thiolated arsenic species.

Methylation has long been considered a detoxification pathway, with As3mt being the primary enzyme for the methylation of arsenic. As3mt is widely distributed in tissues, such as liver, kidney, testis, and lung,[25] and plays a key role in arsenic metabolism and toxicity, as revealed by a few studies using As3mt knockout mice.[48−51] In addition to the methylation catalyzed by As3mt, a high degree of methylation of iAs [10 μg of methylarsenical (g of biomass)$^{-1}$ h^{-1}] and As-contaminated soils [up to 28 μg (g of biomass)$^{-1}$ h^{-1}] by the gut microbiota was also reported.[14] DMAsV is the primary metabolite during arsenic detoxification and is readily excreted in urine. In this study, we discovered that the level of excretion of DMAsV in urine was lower in the treatment group and excretion was typically accompanied by an increased level of inorganic arsenic species, indicating the detoxification was impaired by the gut microbiome perturbations. Because both tissue enzymes and gut microflora are responsible for the methylation of arsenic, any measurement of methylated arsenic species reflects the superimposed effects of both factors. Significantly, modulated DMAsV may therefore result from perturbed gut microbiome communities or changes in the As metabolic capacity of tissues during infection. Previous studies, however, have revealed that gut microbiome changes can also induce different expressions of liver metabolizing enzymes and functions of other organs distant from the gut.[22,43] For instance, colonization of gut bacteria was associated with modifications of hepatic Cyp8b1 expression and the subsequent alteration of bile acid metabolites, including taurocholate and tauromuricholate.[22] The expression and activity of major drug-metabolizing enzymes, such as Cyp3A11 and Cyp2C29, were also significantly stimulated by gut microbiome changes.[22] The systemic effects of the gut microbiome can even reach the brain, and microbial colonization initiates signaling mechanisms that affect neuronal circuits involved in motor control and anxiety behavior.[43] It is therefore also likely that enzyme systems that regulate the metabolism of arsenic could be modulated by gut microbiome perturbations, which in turn contribute to the reduced methylation capacity of the treatment group. Answers to this intriguing question await future experiments.

There is considerable individual variation in arsenic metabolism and toxicity,[52−55] and some individuals methylate and excrete arsenic less efficiently than others, which could place them at higher risks of developing arsenic-induced diseases. Different mechanisms for these variations, e.g., genetic variants, epigenetic regulation, and nutrition homeostasis, have been proposed.[56−58] Genetic susceptibility factors for arsenic-induced diseases have been extensively studied.[59−61] For example, single-nucleotide polymorphisms of four genes, including purine nucleoside phosphorylase (PNP), As3mt, and glutathione *S*-transferase omega 1 and omega 2, which mediate multiple-step metabolism of arsenic through sequential reduction and oxidative methylation, were examined, and three exonic polymorphisms, His20His, Gly51Ser, and Pro57Pro of PNP, were recently found to be associated with arsenicism.[60] The abundance and patterns of arsenic species have long been closely associated with toxicity in individuals exposed to arsenic, with the MMAsV/DMAsV ratio showing a positive association with arsenic toxicity in several studies.[61,62] In particular, large

individual variations in the gut microbiome and metagenomic genotypes have been reported[63−65] In this complementary and specific study, we showed that infection-induced gut microbiome perturbations affect the spectra of arsenic metabolites, thus implicating the gut microbiome as a potential risk factor associated with individual susceptibility to arsenic exposure.

We have also demonstrated that the infection and/or gut microbiome has a dynamic effect on the spectra of arsenic metabolites (Figure S4 of the Supporting Information), which may reflect the temporal establishment of the gut microbiome communities following infection. It is well documented that infants acquire their gut microbiome from the environment through interaction with their mothers[66] and that the establishment of the gut microflora is a temporal process, with bacterial abundances increasing ∼6 orders of magnitude within weeks of life and becoming more adultlike within the first year of life.[41] Similarly, animal studies also show that reconstruction of the gut microbiota ecosystem in germ-free mice through colonization of gut bacteria is a dynamic event, and gut microbiome compositions in colonized mice are highly time-dependent.[22] In particular, we discovered that the formation of DMTAsV, a gut flora-generated arsenic metabolite, is time-dependent, highlighting the dynamic effects of gut microbiome changes on the biotransformation of specific arsenic metabolites.

Measuring arsenic species in urine only represents a limitation of the study, because these data do not reveal the retention and/or redistribution of As species in tissues. Future studies are needed to pinpoint the effect of bacterial infection on the tissue arsenic burden. In addition, we took advantage of an animal model with a perturbed gut microbiome caused by bacterial infections to emulate naturally occurring and universal events in human exposure scenarios. Although it has been demonstrated that C57BL/6 mice are not susceptible to *Helicobacter* spp. and the infection-induced immune response is limited, it would be challenging to completely rule out the role of the immune response in regulating arsenic biotransformation, because the immune system is intrinsically intertwined with the gut microbiome. Animal models established via other approaches, such as transplantation of the cecal microbiota, may serve as better tools for further elucidating the role of the gut microbiome in mediating arsenic metabolism in future studies.

In summary, we have combined an animal model with altered gut microbiome, 16S rRNA gene sequencing, and arsenic speciation to analyze the impact of gut microbiome changes on the biotransformation of arsenic. Several arsenic species were significantly modulated by gut microbiome perturbations resulting from bacterial infection, and a dynamic effect of the gut microbiome on the metabolism of arsenic was also revealed. Taken together, these data show that gut microbiome compositions play a role in defining the abundance of specific toxic species, supporting the hypothesis that gut microbiome phenotypes affect the spectra of arsenic metabolites. The finding that bacterial infection impacts the biotransformation of arsenic may improve our understanding how infectious diseases and environmental exposure interact. Moreover, this study highlights the gut microbiome phenotype as a potential novel risk factor associated with individual susceptibility to diverse environmental chemicals.

■ ASSOCIATED CONTENT

Supporting Information

Abundance of gut bacteria at the phylum level of each sample revealed by 16S rRNA sequencing; histological analysis of the controls and infected animals, with inflammation being scored for multiple regions of the colon and liver; correlation analysis between iAsV with methylated (DMAsV) and thiloated arsenic species (DMTAs) in 1 day exposure samples; and dynamic interaction between the gut microbiome perturbations and arsenic species. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

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■ ABBREVIATIONS

ICP-MS, inductively coupled plasma mass spectrometry; SAM, *S*-adenosylmethionine; iAs, inorganic arsenic; MMAsV, monomethylarsonic acid; MMAsIII, monomethylarsonous acid; DMAsV, dimethylarsinic acid; DMAsIII, dimethylarsinous acid; MMAsV, monomethylarsonic acid; DMTAsV, dimethylmonothioarsinic acid; MMMTAsV, monomethylmonothioarsonic acid; AsB, arsenobetaine; TMAsO, trimethyl arsine oxide; QIIME, quantitative insights into microbial ecology; OTUs, operational taxonomic units; RDP, ribosomal database project; PCoA, principal coordinate analysis; UPGMAD, unweighted pair group method with the arithmetic mean; DMDTAs, dimethyldithioarsinic acid; As3mt, arsenic (+3 oxidation state) methyltransferase; DMDTAs, dimethyldithioarsinic acid

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