Gut Microbiome and Health Assessment Due To Arsenic Toxicity: A Review

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Abstract: Arsenic is considered as a class 1 carcinogen and first among toxicants ranked by the Environmental Protection Agency. Arsenic toxicity includes deleterious effect on gut microbiota, gastrointestinal disorder, immunological disturbances, disrupting metabolism and compromising the host health. Over 10^7–10^9 microorganisms with possibly 500 to 1,000 different species inhabit within the gut with 150 times more genes than the human genome. They help to digest food and play an essential role in our well-being. Gut microbiota affects our whole metabolism as well as the immune system of the host. Arsenic induced toxicity is a major health challenge leading to many neurological and immunological problems and inhibits the growth of many bacterial species common in the gastrointestinal tract. The Gut microbiome carries multiple functions that are beneficial to the hosts. Arsenic exposure will be a critical concern for human health. Human gut microbiomes may be biochemically responsible for arsenic metabolism, change in the arsenic compounds and several arsenical transformations that may lead to arsenic toxicity. Arsenic metabolism occurs in the liver by arsenic methyltransferase (AS3MT) which methylates it into the inorganic arsenic, and ultimately eliminated through urine. Recent studies showed that biotransformation of gut microbiome causes alteration of microbiome morphology and physiology that may alter the ArsBC gene activity due to arsenic toxicity. We aimed at summarising that arsenic induced perturbed gut microbiome communities that trigger systemic responses in diverse organs. Due to gut microbiota perturbation, changes in gut permeability and metabolism have been identified, and there is a shift in the population of gut bacterial species having arsenic resistant genes that result in disturbance of host metabolic homeostasis. Here we review known aspects of arsenic gut microbes’ interaction, this will help to understand about arsenic toxicity with the gut microbiome and their deleterious effects.

Keywords: Arsenic toxicity, arsenic metabolism, gut microbiome, arsenic-microbe’s interaction, host health assessment.
1. INTRODUCTION

Arsenic ranks first among toxicants, as indicated by the Environmental Protection Agency (EPA) and the Agency for Toxic Substances and Disease Registry (ATSDR).1 Arsenic is ubiquitous in the environment, and humans are thus exposed to inorganic and organic arsenic through medicinal, criminal, environmental and occupational sources.2 More than 80% of arsenic compounds are used to manufacture products with agriculture applications such as insecticides, herbicides, fungicides, algaecides, wood preservatives, dyestuffs, and medicines to eradicate tapeworms in sheep and cattle. However, the primary exposure to inorganic arsenic is the ingestion of high metal contaminated drinking water for the general population. Arsenic has been found in groundwater in West Bengal and Bangladesh, and individuals using such water suffer from arsenicosis.3 The level of arsenic in drinking water is found more than the standard limit (10 μg/L) recommended by the World Health Organization (WHO) and EPA, being toxic to over 200 million people worldwide.4 Arsenic is found in nature as inorganic arsenic compounds by forming compounds with oxygen, chlorine and sulphur.5 Accumulation of inorganic arsenic and methylated arsicals are found in various brain parts, with maximum amount in the pituitary.6 The impact of microbiomes on host health and diseases is well reported. The gut microbiome is maintained by host environments that affect the host's metabolic, immune, and neuroendocrine functions, making it an important pathway contributing to health inequities.7 Report showed that the human gut microbiome may biochemically change the arsenic compounds, and several arsenical transformations by bacteria may lead to arsenic toxicity to the host.8 Arsenic exposure is common through arsenic contaminated water and food, leading to disturbance in gut microbial activities. Microbiome composition and diversity in the gastrointestinal tract (GIT) vary, with individuals playing an essential role in determining the initial fate, mobility and relative toxicity of arsenic, whether inhaled or ingested. Gut microbiome also plays a major role in arsenic redox speciation, which enters GIT by intake of arsenic polluted food and water.9 Toxicity of arsenic is associated with its metabolism from inorganic to organic forms, namely the trivalent species monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA). Inorganic arsenic is more toxic than organic arsenic (MMA, DMA), and arsenite (As (III)) toxicity is greater than arsenate toxicity (As (V)).10 The disease showing symptom variability is may be due to the link between arsenic metabolism via intestinal microbiota, host exposure, and disease possibility.9 The disturbance in the gut microbiome could result in gut dysbacteriosis, gastrointestinal infection, immunomodulation, and neurobehavioral changes.11-13 Therefore, the microbiota of GIT plays a crucial role in the host's health and metabolism, including humans.14 This review summarises the role of gut microbiomes that may influence the arsenic metabolism and variation in toxicity of arsenic susceptibility to the host health [Figure 1].

1.1 Occurrence, distribution and compounds

Arsenic is a metalloid present ubiquitously in nature. Trace amounts are found in soil, water and air; present as sulfides in combination with ores of lead, copper, nickel, antimony, cobalt and iron. The sources of arsenic are volcanic eruption, smelting of metals, fuel combustion and the practice of pesticides. The water acts as a carrier of arsenic in the environment, and it is used in agrochemical products, pharma companies and glass industries.15,17 Arsenic is geologically present in groundwater, making it an integral part of drinking water in most parts of the world. On comparing globally, Bangladesh, India, China and Taiwan report the highest level of arsenic.18 Compounds of arsenic can be categorised into three classes: inorganic, organic, and arsine gas [Table 1].
Inorganic arsenic has two most common oxidation states: trivalent and pentavalent. Inorganic arsenic compounds with trivalent oxidation state including arsenic trioxide, sodium arsenite and arsenic trichloride, while pentavalent oxidation state includes arsenic pentoxide, arsenic acid, and arsenates, eg. lead arsenate (PbHAsO₄) and calcium arsenate [Ca₃(AsO₄)₂]. Arsenic trioxide (CH₃₃AsO₃), methylarsenic acid (CH₃₃AsO₃), dimethylarsinic acid (C₃H₇AsO₃) and arsenobetaine (C₃H₇AsO₃) are usual organic arsenic compounds. Arsenic gas (AsH₃) is colourless and flammable and generated when arsenic containing compounds release nascent hydrogen. Arsenic toxicity is distinct from the toxicity of inorganic or organic arsenic compounds.

### 1.2 Arsenic toxicity

At present, arsenic exposure is still a major health problem. About 140 million people in 50 countries consume arsenic contaminated water above the WHO standard (0.05mg).

Arsenic interferes with general cellular processes like cellular enzymes, cell respiration and mitosis by affecting the system that leads to a disruption in the system that leads to a chain of biological and physiological phenomenon. Arsenic exposure leads to a disruption in the system that leads to a chain of widespread effects. Arsenic exposure has been shown to elevate hypothalamic corticotropin releasing factor (CRF), modified corticosterone (CORT) secretion, reduction in hippocampal hydroxyoxygen dehydrogenase type I (11β-HSD1), and decreased expression of brain derived neurotrophic factor (BDNF). This impairment in the HPA axis leads to molecular and cognitive pathology.

### 1.3 Absorption, accumulation and methylation

Arsenic ingestion in human body occurs via food and water, which is absorbed mainly through GIT even at a low dose. The uptake of almost 90% of soluble arsenic is in inorganic trivalent or pentavalent forms. After absorption in the stomach and intestine, it is released into the bloodstream and accumulates in many parts of the brain, muscles, bones, kidneys and lungs in the form of inorganic and methylated arsenicals.

Flow of arsenic from the blood seems to follow a three-compartment model, which speculates biomethylation of inorganic arsenic. In humans, data based on autopsy indicates the highest concentration of arsenic in skin, nails and hair. Arsenic distribution in the organs shows 2-25 times greater in the kidneys, liver, bile, brain, skeleton, skin, and blood for trivalent than pentavalent forms. Arsenic is detoxified through a process called methylation. It is cycling in any environment, and human exposure is recently considered a bioactivation and detoxification pathway and is directly related to its chemical speciation.

Methylation materialises through alternating reductive and oxidative methylation reactions; the addition of methyl group (CH₃) is the main factor for reducing pentavalent to trivalent arsenic. Methylated MMA and DMA are relatively less toxic, having less binding capacity to tissues and greater elimination from the body than unmetylated forms. Arsenic metabolism occurs in the liver. Arsenic methyltransferase (AS₃MT) methylates the inorganic arsenic in liver in the presence of a methyl donor S-adenosylmethionine (SAM) and a cofactor glutathione (GSH) to significant monomethylated (MMA) and dimethylated arsenic before excretion through urine.
Reports showed that the gut microbiome of humans is directly involved in the arsenic reduction/oxidation methylation pathway. In this process, inorganic arsenic (As V), on reduction, converts into more toxic inorganic arsenic (As III), which on methylation and oxidation transforms into MMA\(^V\) simultaneously. On reduction of MMA\(^V\), MMA\(^III\) is formed, which on oxidative methylation converted into DMA\(^V\), and further reduced to DMA\(^III\).\(^{36-37}\)

### 2. Arsenic-Microbe’s interaction

Arsenic-microbes interaction derives from environmental microbiology, where microbial metabolism is the major factor of arsenic speciation, mobility, and toxicity.\(^8\) In all environments, microbes are the leading carriers, where arsenic and microbes coexist together and thus structural part of arsenic cycling for arsenic transformation. Arsenic is transformed by oxidation, reduction, or demethylation based on microbial catalysts.\(^31\) All the reactions carried out by microbes are of self-interest as may be carried out for detoxification of poison or cellular energy generation.\(^8\) Recent studies showed that due to arsenic toxicity, gut microbiome biotransformation cause alteration of microbiome morphology and physiology that may directly alter the ArsBC gene activity.\(^8\) Although ingested arsenic come first in contact with the GIT and effect resident bacteria. Orally ingested arsenic is detoxified and removed from the body by gut microbiomes by affecting their metabolism and making the novel arsenic metabolites accessible to the host.\(^38\) Toxicant killing of microbe members may alter the maintenance of the gut epithelial barrier, regulate host inflammatory responses, and synthetise or recycle essential metabolites and cofactors involved in the host toxic responses pathway.\(^8\) Exposure of environmentally relevant concentrations of arsenic in drinking water impact the microbial community of the colon to alter both microbiome and host metabolism.\(^12\)

#### 2.1 Effect on gut microbiome

Several studies showed that due to arsenic, the constitution of the microbiome community is changed. In the context of phylogenetic examining studies, fish, rodents, and ruminants are compatible in representing arsenic induced disrupting microbiome community constitutions.\(^39,40\) Arsenic exposure leads to alteration of microbiome composition in the gut because of various factors, including variable arsenic tolerance and detoxification capacity, e.g. Methyl Arsenite efflux permease (ArsP), non-heme iron-dependent type-I extradiol dioxygenase with lyase activity of C-As bond (ArsI) or methyl arsenite oxidase (ArsH), among genera and species, arsenic–based antibiotics production, e.g. nourseothricin;\(^41-43\) and those changes could have developed arsenic resistance acquired from spontaneous mutations. This may modify host metabolism that, in turn, alter gut microbial community.\(^44\) Reports suggested that mice exposed to arsenic results in elevation of Bacteroidetes population.\(^12\) Elevated Bacteroidetes number may be due to gram-negative bacteria as they have lipopolysaccharide (LPS) on their outer membrane. The LPS cause widespread inflammation, disrupting normal biological functions, and it is an important virulence factor.\(^45\) Increased concentrations of pathogenic arginine metabolites have been detected in the arsenic exposed mouse circulation. Intracellular pathogens such as Salmonella typhimurium increase the level of arginine metabolites by utilising the arginine pool of the host on disruption of normal biological functions, and it is an important virulence factor.\(^45\) The New Hampshire Birth Cohort Study (NHBCS) showed the link between arsenic exposure and gut microbiome constitution in over 1500 pregnant women (18–45 ages) and subsequently in their offsprings. In 204 arsenic exposed six week old infants, urinary samples showed arsenic concentration below the quantification limit of 4.8μg/L and suppressed microbiome composition of several genera (Firmicutes, Bacteroides, and Bifidobacterium). They reported sex-dependent differences in the infant gut microbiome composition, leading to perturbation in the gut microbiota community.\(^47\) Six week old female C57Bl/6 mice showed variation in the various intestinal flora, leading to clear β-
diversity clustering between treated and control individuals, exposing arsenic resulting decrease in order *Streptophyta*, *Clostridiales*, and *Erysipelotrichales*, whereas *Bacillales* were increased in arsenic exposed individuals. Stool microbiome samples of Bangladesh children were analysed who were exposed to arsenic contaminated drinking water and found modified microbiome constitutional shifts, with elevated copy number of *Proteobacteria* and arsB and arsC on increasing arsenic exposure.

3. **Arsenic toxicity to Gut microbiome and host health**

The gut microbiome impacts various biological functions, including metabolic processes, energy cycle, and immune system development. In the human body, cells and gut microbiome due to stored arsenic within them, harmful effects are ensured. Disruption of gut microbiome composition due to arsenic exposure results in host diseases. Bacterial communities are susceptible to altering the host surrounding and thus disturbance in gut microbiomes and secretion of virulence factors such as LPS due to microbiome shift favouring more infective bacterial species, cause an increase in pathogenic arginine metabolites and may be responsible for originating host diseases. Advancement of liver fibrosis and hepatocarcinoma are induced by the change in gut microbiome permeability and rise in pathogen-associated molecular patterns (PAMPS) such as LPS. Moreover, arsenic exposed at a higher dose increases the chances of mitochondria damage to impair energy metabolism and cause cell death. Altered gut microbiome constitution and its functions are associated with oxidative stress and many pathological diseases like diabetes, inflammatory bowel disease (IBD), cancer, Parkinson's disease, cardiovascular diseases, allergies, and inflammatory diseases occurring in hosts due to dysbiosis. [Table 2]

<table>
<thead>
<tr>
<th>Animal Model</th>
<th>Dose of arsenic exposure</th>
<th>Major findings</th>
<th>References</th>
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<tr>
<td><strong>Mice</strong></td>
<td>Arsenic trioxide (10, and 250 ppb) for 2, 5, and 10 weeks</td>
<td>Arsenic concentration changed the diversity of bacteria at both genetic and morphological levels, especially within two bacterial phylum <em>Bacteroidetes</em> and <em>Firmicutes</em>. The level of arsC metabolites was found elevated in the blood. Histopathological study of the liver revealed increased nitrate and nitrite levels at a higher dose and decreased bacterial colonies.</td>
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<td><strong>Larvae zebrafish</strong> 20 days post fertilisation (dpf)</td>
<td>Arsenic compound (10, 50, and 100 ppb) exposure for 20 days</td>
<td>Arsenic alters the microbial composition, diversity and causes dysbiosis, and at higher doses increase the level of class 1 integron gene in developing larval zebrafish microbiota. The higher concentration causes increased expression of the int gene (1 integron gene), which is responsible for horizontal transfer of resistance gene and even at the lowest concentration, there was destabilisation in bacterial colonies.</td>
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<td><strong>Mice C57BL/6</strong></td>
<td>Fed with 10ppm arsenic compound in drinking water for four weeks</td>
<td>The 16S rRNA sequencing after a particular exposure of arsenic revealed imbalanced homeostasis of the host and changed significantly gut microbiota.</td>
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<tr>
<td><strong>Mice C57BL/6</strong></td>
<td>One group of mice fed with 50 ppm cadmium chloride and another group with 50 ppm sodium arsenite for two weeks as drinking water</td>
<td>16S rRNA gene amplicon sequencing and untargeted LC-MS/MS metabolomics indicate that bacterial diversity in cadmium was much lower compared to arsenic both quantitatively and qualitatively.</td>
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<td><strong>Mice C57BL/6</strong></td>
<td>Exposed with 0, 50, and 500 ppb of arsenic with zinc adequate and with 0, 50, and 500 ppb of arsenic with marginally zinc-deficient for six weeks in fresh drinking water</td>
<td>This study was carried out to observe the individual and combined effect of two metals, zinc, and arsenic on the gut microbiota of mice. The zinc was restricted in the diet of mice while arsenic was provided; this resulted in reduced diversity of bacteria while their combined effect modified the diversity. There was also a decrease in zinc levels in plasma, and DNA damage was also reported.</td>
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<tr>
<td><strong>Pathogen-free grade C57BL/6 female mice</strong></td>
<td>Treated with 100ppb of sodium arsenite for 13 weeks in drinking water</td>
<td>16S rRNA sequencing data showed that the overall diversity of bacteria was reduced. The expression of genes related to carbohydrate metabolism decreased while lipopolysaccharide synthesis gene, DNA repair gene, and stress responsive gene expression increased after treatment. The expression level of genes related to the synthesis of vitamins B6, B12, and K2 and folic acid elevated.</td>
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<tr>
<td><strong>Wild-type and IL-10 gene knockout mice</strong></td>
<td>Arsenic compound exposure (10ppm) for four weeks in drinking water</td>
<td>16S rRNA gene sequencing and HPLC-ICP-MS data showed that due to the absence of IL-10 gene, the rate of infection was higher in the host's gut, which in turn affects the composition of bacteria and aswell as arsenic metabolism.</td>
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3.1 Gastrointestinal tract disorders

In the GIT, high exposure to arsenic causes gastric mucosal hyperaemia and haemorrhagic injury, whereas low and moderate arsenic level alters cell signalling that regulates cell differentiation and functions. Reports suggest that moderate level of arsenic exposure (250 ppb) degrade intestinal microbial biofilms that increase bacterial spores, reduce intracellular inclusion and alter gut microbiome leading to modified physiological functions resulting in the potential opening of niche for pathogenic microbes like, *Bacteroidetes* which may cause GIT related disorders and inflammation. Intestinal microbes are responsible for converting organic arsenic to harmful inorganic arsenic, altering certain microbial population viability. When 50 ppm arsenic is exposed to mice results in variation in microbiome metabolic profile by altering some gut microbial family’s abundance suggested by the metagenomic study.

3.2 Immunological disturbances

The infectious agents of pathogenic bacteria trigger immune system alterations leading to several immunological disturbances. Dysfunction of mucosal barrier is caused by bacterial penetration product resulting in direct contact of immune cell. Mechanisms implied by chronic microbiome change for disease growth are similar to vascular and metabolic disease, including dysfunctional metabolism, altered lipid - deposition, and chronic inflammation. Inflammatory dendritic cells (DCs) promote the secretion of proinflammatory, inflammatory chemokines and prostaglandins, IL-17 produce TNF-α and IL-6 leads to the Th17 cells, which caused inflammation and tissue destruction, which are responsible for various many immune-inflammatory diseases. Population-based study reveals that arsenic exposure in human placenta and placental blood is related to oxidative stress, inflammation, and immune disruption. Repeated exposure of arsenite to adult mice cause an abundance of bacteria belonging to genera *Alistipes*, *Bilophila* (causative agent for inflammatory bowel syndrome (IBD)) and *Lactobacillus johnnii*. The production of IL-12 was enhanced in arsenic exposed mice mediated by *Lactococcus lactis*. Arsenic toxicity can contribute to the intestinal microbiota composition and gut-associated immune response. Repeated arsenic exposure caused a transient increase in the recovery of intestinal bacteria, a shift in the bacterial population with an abundance of arsenic resistance genes, and evidence for host metabolism of arsenite into less-reactive trivalent methylated species. In adult CD-1 mice, arsenic induced a high level of CC chemokines and proinflammatory disturbances in the epithelial lining of the gut. Mice infected with *Helicobacter* trignonum exposed to arsenic alters gut microbiota showed significant changes in the number and regulatory pattern of the metabolites. Another major pathway perturbed due to arsenic exposure after gut microbiome alteration in phospholipid metabolism in the host, followed by sphingolipid, fatty acid metabolism, cholesterol biosynthesis and metabolism, and tryptophan metabolism. This indicates that changes in the gut microbiome exacerbate arsenic toxicity.

4. CONCLUSION

The present review focus on the influence of arsenic toxicity on the gut microbiome. Disturbance in the gut microbiome leads to a variety of challenges to human health. Under the arsenic microenvironment, the cell membrane of intestinal bacteria may absorb the arsenic via ion channels thus, making it less available to the host, and it may lead to perturbations of gut microorganisms. Further, exposure to arsenic also leads to epigenetic changes in the host, deleterious effects on gut microbiota, endocrine disruption, inhibition of DNA repair, and also affect embryonic development and modification of cellular signalling via altered activation of transcription factors. It acts as an indicator of microbial perturbation and infection. The available data on arsenic microbe interaction suggests that gastrointestinal tract disorder, disruption of metabolic function, and immunological disturbances collectively provide mechanistic insight suggesting that the disturbance in the composition of the microbial profile of gut microbiota directly impacts the host health. However, the precise molecular mechanism of arsenic-induced toxicity on human health is still under investigation. Therefore, more focus will be required to understand the interactions among arsenic toxicity, gut microbiome and host health.

5. AUTHORS CONTRIBUTION STATEMENT

Abhishek Jain and Dr. Subodh Kumar Jain conceptualised and prepared the final manuscript. Swati Jain, Roshni Jain and Swati Singh Thakur helped in the collection of research papers during literature survey. All authors discussed the literature surveyed and helped in writing the manuscript.

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7. CONFLICT OF INTEREST

Conflict of interest declared none.

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