Review Article

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Gut microflora & toxic metals: Chromium as a model

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The gastrointestinal tract (GIT) is exposed to various environmental pollutants including metals, that contaminate food and water which may have toxic effects on body. GIT has large amount of microbes that live in symbiosis and help the host in different ways. The resident gut microflora have a significant role to play in detoxification and elimination of the harmful metals from the body. Chromium is a naturally occurring heavy metal found commonly in environment in trivalent (Cr III) and hexavalent (Cr VI) forms. Cr (VI) compounds have been shown to be potent occupational carcinogens. The reduction of Cr (VI) to Cr (III) results in the formation of reactive intermediates that together with oxidative stress and oxidative tissue damage, and a cascade of cellular events including modulation of apoptosis regulatory gene p53 contribute to the cytotoxicity, genotoxicity and carcinogenicity of Cr(VI)-containing compounds. The data discussed here with reference to chromium show that gut microflora have a marked capacity to cope with the increased load of ingested metals and may contribute significantly in the protection against metal toxicity.

Key words Chromium (VI) reduction - chromium resistance - *Escherichia coli* - gut microflora - intestinal bacteria - *Lactobacillus* Prebiotic - probiotic - *Pseudomonas* - toxic metals

The gastrointestinal tract (GIT) is exposed to different environmental pollutants that contaminate food and water. These include metals that may have toxic effects on body. Many metals have no known biological function and some of these are capable of disrupting essential physiological processes. Examples include arsenic, cadmium, lead, chromium and mercury. Some metals also serve a chemically important role as essential components of many enzymes. These metalloenzymes are involved in the synthesis, repair and degradation of biological molecules, release and recognition of certain biological signaling molecules, and transfer of small molecules and electrons in crucial processes such as in respiration. For example, iron-containing haemoglobin transports oxygen in blood. The toxic effects of most metals can be traced due to their ability to disrupt the function of essential biological molecules, such as proteins, enzymes and DNA. In some cases this involves displacing chemically related metal ions that are required for important biological functions such as cell growth, division and repair.

Certain heavy metals form very stable and long-lasting complexes with sulphur in biological molecules, which can disrupt their biological function. In some cases these metals may be concentrated at higher levels of the food chain. The body has developed various mechanisms to detoxify the toxic substances, including the metals and the cells and the secretions of GIT play an important role in this process. The cells have evolved a complex network of metal trafficking pathways. The object of such pathways is to prevent accumulation of the metal in the freely reactive form (metal detoxification pathways) and to ensure proper delivery of the ion to target metalloproteins (metal utilization pathways)[1]. In recent times, microbes have been shown to reduce a wide range of toxic metals *viz*., chromium [Cr(VI)], mercury [Hg(II)], cobalt [Co(III)], lead [Pb(II)], and

arsenic [As(V)]. Under certain conditions, microbial metal reduction can mobilize toxic metals with potentially calamitous effects on human health. Lloyd[2] has discussed the role of microbes in reducing different metals and its impact on the environment. The resident gut microflora may also have a significant role to play in detoxification and elimination of the harmful metals from the body. This review covers the existing knowledge on this aspect with reference to chromium.

Gut microflora: A large number of bacteria belonging to 300–500 different species live and grow in the human intestine as symbionts[3-4]. Some common resident bacteria found at different locations in the GIT are listed in the Table. The stomach, duodenum (0-10^4 bacteria/g of the luminal contents) and small intestine (10^5-10^6 bacteria/g) contain smaller number of bacteria adhering to the epithelia and some other bacteria in transit. This may be because of the composition of the luminal fluid containing acid, bile, and pancreatic secretion, which kill most ingested microorganisms. On the other hand, the large intestine contains a complex and dynamic microbial population with high densities of living bacteria. The luminal contents may have up to 10^{11} or 10^{12} bacteria/g[3]. Some of these bacteria are potential pathogens and can be a source of infection and sepsis under certain conditions, for example when the integrity of the bowel barrier is physically or functionally broken down. A constant interaction between the host and the microbes provide important health benefits to the human host[5].

Microorganisms start colonization of the gastrointestinal tract soon after birth and this process continues throughout the life. The environmental factors have a major role in determining the extent and type of colonization, for example, differences exist between people living in developed countries and those in developing countries[6-7]. Some bacteria can modulate expression of genes in host epithelial cells[8], thus creating a favourable habitat for themselves, and can prevent growth of other bacteria introduced later. The initial colonization is therefore very relevant to the final composition of the permanent flora in adults. It has been shown[3] that anaerobic bacteria outnumber aerobic bacteria by a factor of 100–1000. The predominant genera in human beings are *Bacteroides, Bifidobacterium, Eubacterium, Clostridium, Peptococcus, Peptostreptococcus,* and *Ruminococcus etc*.[3,5], followed by aerobes (facultative anaerobes) such as *Escherichia, Enterobacter, Enterococcus, Klebsiella, Lactobacillus, Proteus etc*. (Table). Every individual has several hundreds of species, with a particular combination that is distinct from that found in other individuals. The species vary greatly between individuals[3,9].

Molecular biology techniques are being used to investigate the microbial ecology in the gut without use

Table. Common resident gut microflora

Part of GIT	Some common resident bacteria
Mouth and oropharynx	*Streptococcus viridians, Streptococcus pneumoniae,* Beta-haemolytic streptococci, coagulase-negative Staphylococci, *Veillonella spp., Fusobacterium* spp., *Treponema* spp., *Porphyromonas* spp., *Prevotella* spp., *Neisseria* spp. and *Branhamella catarrhalis, Candida* spp., *Haemophilus* spp., *Diphtheroids, Actinomyces* spp., *Staphylococcus aureus, Eikenella corrodens*
Stomach	*Streptococcus, Staphylococcus, Lactobacillus, Peptostreptococcus*
Small intestines	*Lactobacillus* spp., *Bacteroides* spp., *Clostridium* spp., *Mycobacterium* spp., Enterococci, bacteria of Enterobacteriaceae,
Large intestines	*Bacteroides* spp., *Fusobacterium* spp., *Clostridium* spp., *Peptostreptococcus* spp., *Escherichia coli, Klebsiella* spp., *Proteus* spp., *Lactobacillus* spp., Enterococci, *Streptococcus* spp., *Pseudomonas* spp., *Acinetobacter* spp., coagulase-negative Staphylococci *Staphylococcus aureus, Mycobacterium* spp., *Actinomyces* spp., *Bifidobacterium bifidum, Enterobacter* spp., *Peptococcus* spp., *Methanogens* (Archaea), *Salmonella* spp.

GIT - Gastro intestinal tract

of cultures[10]. These studies show that many DNA sequences correspond to previously undescribed microorganisms, and also that every individual has unique strains of bacteria[11,12]. Molecular procedures have shown that aerobes, including *Escherichia coli*, *Enterococci* and *Lactobacilli* achieve very high densities and metabolic activity in the human caecum[13]. Use of animals bred under germ free conditions[14] suggests that gut microflora have a number of important functions in the body.

Protective functions: Resident bacteria are a crucial line of defence to colonization by exogenous microbes and, therefore, are important in prevention of invasion of tissues by pathogens. Germ free animals are very susceptible to infection[15,16]. This is also relevant to opportunistic bacteria that are present in the gut but have restricted growth. The equilibrium between species of resident bacteria provides stability in the microbial population within the same individual under normal conditions. However, use of antibiotics can disrupt the ecological balance and allow overgrowth of species with potential pathogenicity[17]. Several mechanisms have been implicated in the barrier effect, for example, competition for attachment sites on intestinal epithelial cells and for nutrient availability[18-19]. These bacteria can inhibit the growth of their competitors by producing antimicrobial substances known as bacteriocins[20-21].

Role as probiotics and prebiotics: Probiotic is a bacterium that provides specific health benefits when consumed as a food component or supplement. They are living microorganisms that upon ingestion in specific numbers, exert health benefits beyond those of inherent basic nutrition[22]. According to this definition, probiotics do not necessarily colonize the human intestine. The effect of a bacterium is strain specific and cannot be extrapolated even to other strains of the same species. The prebiotics are non-digestible food ingredients that benefit the host by selectively stimulating growth, or activity, or both, of one or a restricted number of bacteria in the colon[23].

Orally administered probiotics can enhance specific IgA responses to rotavirus in infected children[24] or to *Salmonella* Typhi in adults undergoing vaccination with an attenuated strain[25]. In healthy people, two different probiotics administered in a fermented milk product transiently colonized the gut and enhanced phagocytic activity of circulating leucocytes for a few weeks while colonization persisted[26]. Probiotics and prebiotics have been shown to prevent colon cancer in several animals, but their role in reduction of risk of colon cancer in human beings is not established[27]. However, probiotics have been shown to reduce the faecal activity of enzymes known to produce genotoxic compounds that act as tumour initiators in human beings[28].

Current clinical and animal model studies[29] support a role for certain probiotics, activating the common mucosal system through the stimulation of gut antigen-presenting cells to both promote protection and to switch regulatory mechanisms. It is concluded that a new term is required to identify bacteria that promote health through driving mucosal immune mechanisms, compared to those with strictly local effects. The term immunobiotics has been suggested as appropriate to fulfil this need[29].

Metabolic functions: A major metabolic function of the gut microflora is fermentation of non-digestible dietary residue and endogenous mucus produced by the epithelia[14]. Genetic diversity of the microbes provides various enzymes and biochemical pathways that are distinct from the host's own constitutive resources. This results in recovery of metabolic energy and absorbable substrates for the host, and supply of energy and nutritive products for bacterial growth and proliferation[30-31]. Anaerobic metabolism of peptides and proteins by the microflora produces short-chain fatty acids and a series of potentially toxic substances including ammonia, amines, phenols, thiols, and indols[32]. Colonic microorganisms also play a part in vitamin synthesis[33-34] and in absorption of calcium, magnesium, and iron. Absorption of ions in the caecum is improved by carbohydrate fermentation and production of short-chain fatty acids, especially acetate, propionate, and butyrate[14,35,36]. Epithelial cell growth and differentiation is also affected by gut-microflora. Studies suggest that intraluminal bacteria affect the proliferation and the differentiation of epithelial cells in the colon[8,37].

The intestinal mucosa is the main interface between the immune system and the external environment, which is reflected by the gut-associated lymphoid tissues containing the largest pool of immunocompetent cells in the human body[38]. Animals bred in a germ free

environment have low densities of lymphoid cells in the gut mucosa and circulating concentrations of immunoglobulins in the blood are low[10,39]. Microbial colonization of the gastrointestinal tract affects the composition of gut associated lymphoid tissue. Many and diverse interactions between microbes, epithelium and gut associated lymphoid tissue are involved in modeling the memory mechanisms of systemic immunity[39].The immune response to microbes relies on innate and adaptive components, such as immunoglobulin secretion[40].

Role of microflora in colon cancer: Intestinal bacteria can play a part in initiation of colon cancer through production of carcinogens, cocarcinogens, or procarcinogens. Some intestinal microbes strongly increase damage to DNA in colon cells induced by heterocyclic amines, whereas other intestinal bacteria can uptake and detoxify such compounds[41]. Bacteria of the *Bacteroides* and *Clostridium* genera increase the incidence and growth rate of colonic tumours induced in animals, whereas other genera such as *Lactobacillus* and *Bifidobacteria* prevent tumorigenesis[42-44]. Although the evidence is not conclusive, colonic flora seem to be a major environmental factor that modulates risk of colonic cancer in human beings.

Chromium: Chromium (Cr) is a heavy metal which is found in the environment commonly in hexavalent (Cr VI) and trivalent (Cr III) forms. Cr (VI) is a widely used industrial chemical, extensively used in paints, metal finishes, steel including stainless steel manufacturing, alloy cast irons, chrome and wood treatment. The major non-occupational source of chromium for humans is food such as vegetables, meat, urban air, and hip or knee prostheses *etc*.[44,45]. Non-occupational exposure to the metal occurs via the ingestion of chromium containing food and water, whereas occupational exposure occurs via inhalation. Cr(III) is poorly absorbed, regardless of the route of exposure, whereas Cr (VI) is more readily absorbed[46]. Animal studies showed that Cr (VI) was generally more toxic than Cr(III), but neither of the two states was very toxic by the oral route[44,45]. Chromium is localized in the lung, liver, kidney, spleen, adrenals, plasma, bone marrow, and red blood cells[46,47]. Cr(VI) undergoes enzymatic reduction, resulting in the formation of reactive intermediates and Cr(III)[46]. The main routes for the excretion of chromium are via the kidneys/urine and the bile/feces[48]. The respiratory and dermal toxicity of chromium is well documented. Workers exposed to chromium develop nasal irritation, nasal ulcers, perforation of the nasal septum[46,47] and hypersensitivity reactions and "chrome holes" of the skin[49,50]. Among the general population, contact dermatitis has been found to be associated with the use of bleaches and detergents[51]. Compounds of both Cr(VI) and Cr(III) induce developmental defects in experimental animals including neural tube defects, malformations, and foetal deaths[52,53]. The inhalation of chromium compounds has been shown to be associated with the development of cancer in workers in the chromate industry[54]. There is evidence for an increased risk of developing nasal, pharyngeal, and gastrointestinal carcinomas[46] due to cromium exposure.

Cr(III) compounds are also used as micronutrients and nutritional supplements and have been demonstrated to exhibit a significant number of health benefits in animals and humans[55]. Chromium plays an important role in glucose and cholesterol metabolism and is thus essential for man and animals. Chromium is an essential nutrient required by the human body to promote the action of insulin in body tissues so that the body can use sugars, proteins and fats. Chromium polynicotinate, chromium chloride and chromium picolinate have been used as nutritional supplement; control blood sugar in diabetes and may reduce cholesterol and blood pressure levels. Chromium increases insulin binding to cells, insulin receptor number and activates insulin receptor kinase leading to increased insulin sensitivity[48,55]. It also has beneficial effect on both muscle strength and body composition[56].

Toxic effects of chromium: Various metals are responsible for many biochemical, immunological and physiological essential activities of the body as micronutrients but some of these can give rise to disordered functions resulting in increased susceptibility to infections, a variety of hypersensitivity reactions and neoplasia. Cr (VI) is highly toxic to all forms of living organisms and is mutagenic in bacteria[57]. Cr (VI) is very toxic by dermal and inhalation route and causes lung cancer, nasal irritation, nasal ulcer, hypersensitivity reactions and contact dermatitis[44,45,58]. The mechanism of the Cr (VI)-induced cytotoxicity is not entirely understood. A series of *in vitro* and *in vivo* studies[59-61] have demonstrated that Cr (VI) induces an oxidative

stress through enhanced production of reactive oxygen species (ROS) leading to genomic DNA damage and oxidative deterioration of lipids and proteins. A cascade of cellular events occurs following Cr(VI)-induced oxidative stress including enhanced production of superoxide anion and hydroxyl radicals, increased lipid peroxidation and genomic DNA fragmentation, modulation of intracellular oxidized states, activation of protein kinase C, apoptotic cell death and altered gene expression[59-61]. Some of the factors in determining the biological outcome of exposure to chromium include the bioavailability, solubility of chromium compounds and chemical speciation, intracellular reduction and interaction with DNA. The chromium genotoxicity manifests as several types of DNA lesions, gene mutations and inhibition of macromolecular synthesis. Further, chromium exposure may lead to apoptosis, premature terminal growth arrest or neoplastic transformation. Chromium-induced tumor suppressor gene p53 and oxidative processes are some of the major factors that may determine the cellular outcome. These approaches have been used to understand the interrelationship between chromium-induced genotoxicity, apoptosis and effects on immune response[59].

Mechanisms of reduction of Cr (VI) to Cr (III): A number of mechanisms have been reported by which Cr (VI) is reduced to Cr (III). *In vitro* and under physiological conditions, ascorbic acid, thiols, glutathione, cysteine, cysteamine, lipoic acid coenzyme A, and coenzyme M reduce Cr (VI) at a significant rate[62]. The *in vitro* reaction of Cr (VI) with glutathione results in the formation of a Cr (V) intermediate that is possibly the form that interacts with cellular macromolecules[63]. DT-diaphorase is a major cytosolic enzyme involved in Cr (VI) reduction[64]. The NADPH-dependent Cr (VI) reductase activity of rat liver microsomes has been attributed to cytochrome P-450, whereas the Cr (VI) reductase activity of rat liver mitochondria is attributed to NADH-ubiquinone oxidoreductase (complex I)[46]. Suzuki *et al*[65], showed that the Cr (VI) reductase reduced Cr (VI) to Cr(III) with at least two reaction steps via Cr (V) as an intermediate. The mechanisms of Cr (VI) reduction in the bacteria need further in depth exploration.

Reduction of Cr (VI) in the body: Chromium enters the body through inhalation, ingestion and to a lower extent through absorption via skin. Cr (VI) is poorly absorbed in the gut, therefore, it is not very toxic when introduced by oral route. All the ingested Cr (VI) is reduced to Cr (III) before entering the blood stream. Cr (III) is unable to enter into the cells but Cr (VI) enters through membrane anionic transporters. Cr (VI) is metabolically reduced to Cr (III) in the cell. Cr (VI) does not react with macromolecules such as DNA, RNA, proteins and lipids. However, both Cr (III) and the reductional intermediate Cr (V) are capable of co-ordinate and covalent interactions with macromolecules[66].

The cells of the immune system form a strong line of defence against foreign substances. A study was undertaken[67] to investigate the capacity of different cells of Wistar rats to reduce potentially carcinogenic hexavalent chromium into less toxic trivalent chromium *in vitro*. It was observed that among the single cell suspensions from the spleen, a peak reduction of 55 per cent was observed with the total spleen cells, 40 per cent with the B-lymphocyte-enriched subpopulation, 10 per cent with T-lymphocytes and 24 per cent with the macrophages. The reduction by splenic and peritoneal macrophages was similar. Total thymocytes reduced 54 per cent of the Cr (VI). Since the most common route of entry of chromium is through drinking water and food, intestinal cells were also investigated. Among the intestinal cells the maximum reduction of 100 per cent was observed with the upper villus cells and 72 per cent with the middle villus cells while reduction was the least (4%) with the crypt cells. The reduction in the intestinal loop *in situ* was 100 per cent. The time taken by each cell type for the peak reduction of Cr (VI) was markedly different[67]. The findings thus showed that the capacity of different cells in the body differed vastly in their capacity and time taken to reduce hexavalent chromium. The efficient handling of Cr (VI) by intestine is due to the presence of a variety of cells and bacteria. Various organs of the body have capacity to reduce Cr (VI)[66,67].

Reduction of Cr (VI) to Cr (III) by microbes: The presence of chromate in the environment inhibits most microorganisms but also promotes the selection of resistant bacteria[68]. Cr (VI) compounds are markedly efffective than those of Cr (III) due to their high solubility in water, rapid permeability through biological membranes

and subsequent interaction with intracellular protein and nucleic acids. The bacteria present naturally in soil and water bodies are exposed to Cr through contamination with industrial effluents, especially from tannery *etc*. The processes by which the microorganisms interact with the toxic metals enabling their removal/and recovery are biosorption, bioaccumulation and enzymatic reduction. Microorganisms have evolved resistance mechanism to select resistant variants to deal with metal toxicity as the result of exposure to metal contaminated environments, which cause coincidental selection for resistant factors for antibiotics and heavy metals.

There are evidences for possible links between heavy metal and antibiotic resistance in a bacteria because these traits are generally associated with transmissible plasmids and the genes are frequently found on the same plasmid[69,70]. Bacterial resistance to chromate can be due to chromosomal mutation or is plasmid-borne[71]. Many bacteria belonging to gerera *Pseudomonas, Aeromonas, Enterobacter, Escherichia, Bacillus, Streptomyces, etc*., can reduce Cr (VI) to Cr (III). In each case, the plasmid bearing strains are approximately 10-fold more resistant to chromate than the plasmid less strains[72]. Under environmental conditions of metal stress, such metal and antibiotic resistant population adopts faster by the spread of R-factors than by mutation and natural selection thus leading to a very rapid increase in their numbers[73]. Microbial resistance to metal ions and antibiotics is a potential health hazard.

Some bacteria present in water and soil develop resistance to chromium on exposure to Cr-containing effluent in their environment[68,74-77]. These bacteria reduce Cr (VI) into Cr (III) and minimize the adverse effects of Cr (VI) on their growth[77]. Further, some of the bacteria bioaccumulate large quantity of Cr and bring down the residual concentration of Cr (VI) in 24 h[78]. Several studies have been done to investigate the effect of Cr on soil and water bacteria resistant to chromium[57,74-79]. In a recent study[78], 16 per cent of the Cr (VI) resistant bacterial strains isolated from tannery effluent had minimum inhibitory concentration (MIC) more than 100 mg Cr (VI)/l. Another study[80] reports that under *in vitro* conditions, Cr-tolerance may depend on the type of media used, the MIC obtained in the rich media are from two to five times higher than in minimal media because heavy metals can be complexed by some components of media, especially organic substances and phosphate. Bacterial sensitivity to metal toxicity is known to depend on their isolation site. In natural bacterial communities, the development of metal resistance is greatly enhanced by the horizontal dispersal of genetic information[81]. Evolution of resistance via such transfer between natural bacterial isolates has been shown to occur *in situ* and also under laboratory conditions[82]. Widespread bacterial reduction of Cr (VI) to the less toxic Cr (III) ions is well known[79-83]. In different bacteria, chromate reduction is either an aerobic or an anaerobic process (but not both) and is carried out either by soluble proteins or by cell membranes[81].

The ability of microorganisms to alter their chemical physiology in order to compensate for potentially traumatic changes in their external environment represents a built in factor of safety for biological survival. Growth of *E. coli*, *Micrococcus luteus* and *Azotobactor* spp. in the presence of lead and growth of *Chlamydomonas reinhardi* in the presence of mercury are examples of biological accommodation[84-88]. Growth stimulation of facultative *Methylobacterium* spp. following the addition of molybdenum or tungsten, which enhances the intracellular formate dehydrogenase activity, has also been reported[89]. Enzymatic reduction of Cr (VI) by hexavalent Cr-tolerant *Pseudomonas ambigua* G-1 isolated from activated sludge has been reported. The intracellular reducing enzyme required NADH as a hydrogen donor[71]. A membrane-associated chromate reductase activity from *Enterobacter cloacae* isolated from activated sludge has also been documented[75]. In the present context of Cr interaction with facultative gut bacteria further studies are required to identify cellular and molecular mechanisms of accommodation/adaptation as well as stimulation of growth of Cr-stressed *Preudomonas* and *Lactobacillus* spp. Some bacteria are known to bioaccumulate up to 34 mg Cr/g dry weight[78]. Intracellular accumulation of such large amount of Cr may disturb the normal functioning of the bacterial cell. Several factors including mutagenic potential of chromium, a Cr (VI) reductional intermediate product such as Cr (III), a reaction to accumulated Cr or a selective stimulation of the control of binary fission, membrane proteins and lipids interaction and other intracellular mechanisms may play important role. Diverse mechanisms may be responsible for the development of resistance for Cr (VI) in microbes, including chromate efflux[85].

Ingested Cr (VI) and gut microflora: The intestines have a huge population of bacteria (Table) and the caecum harbors the largest number of bacteria[90,91]. Thus, bacteria may play an important role in protecting body from the toxicity of ingested chromium. The resident bacterial flora of the gastrointestinal tract is exposed to Cr through ingestion of water and food contaminated with Cr. It has been reported that human ingestion of Cr (VI) in drinking water at levels of 1 to 10 ppm is safe due to high capacity of gastrointestinal tract to reduce Cr (VI) to Cr (III)[61-92]. In long-term studies, rats were not adversely affected by 2.4 mg/kg/day of Cr (VI) as potassium dichromate in drinking water[92]. A study was undertaken to investigate the effects of chronic ingestion of potassium dichromate [Cr (VI)] on the resident gut microflora of Wistar rats. A group of rats were kept on drinking water containing 10 ppm Cr (VI) (called Cr-stressed animals) and the other group was given plain water. After 10 wk *Lactobacillus*, *Pseudomonas* spp. and *Esch. coli* were isolated from the caecum of the rats. The most significant findings of this study were the stimulation of growth of facultative gut bacteria from the Cr-stressed rats, and also the significant increase of growth even in presence of lower concentrations of chromium. Thus, chromium may act like a prebiotic. Furthermore, the capacity to reduce Cr (VI) was significantly decreased along with the increased tolerance of the bacteria to Cr (higher MIC values), which was associated with the development of antibiotic resistance. The effects were most marked with the *Pseudomonas* spp. and least with the *Esch.* coli. The antibiotic resistance developed in the *Lactobacillus* may be a blessing in disguise as the bacteria may continue to provide benefits even in patients given antibiotic therapy (unpublished data). It appeared that the changes were a sequel to the effort of gut bacteria to provide the first line of defense to the body by converting toxic Cr (VI) to a less toxic Cr (III). Our findings further showed that *Pseudomonas* obtained from the Cr-stressed rat had the highest MIC value while the *Lactobacillus* and *Esch. coli* had lower values. As compared to the bacteria from the normal control rats, the MIC values were significantly higher in the Cr-stressed rats. This indicated that bacteria from Cr-stressed animals tolerated the presence of Cr in the milieu much better. The stimulated growth of Cr-stressed *Pseudomonas* and *Lactobacillus*, and complete Cr (VI) reduction capacity (up to 25 mg/l Cr-concentration) indicated their ability to adaptation. This may also reflect horizontal genetic transfer resulting from Cr-stress. Diversity of Cr-resistant and Cr-reducing bacteria in the bacterial population from a chromium contaminated activated sludge has been established[79]. It has also been suggested that the mechanisms of Cr (VI) resistance and reduction may differ in microbial community from group to group.Therefore, Cr (VI) resistance and reduction capacity could be the shared abilities and not an exclusive characteristic of a single group of bacteria.

A number of components in the intestine may be responsible for the efficient handling of Cr (VI). It has been reported that bacteria isolated from faeces sequestrated 3.8±1.7 ug Cr (VI)/10^9 bacteria; thus the interesting derivation is that 11-24 mg Cr (VI) can be eliminated daily with faeces[66,68]. Further, intestinal bacteria contain high amounts of Glutathione (GSH) which efficiently reduces Cr (VI). Enterobacterial enzymes, such as nitroreductases can also reduce Cr (VI)[93,94]. We have also shown that Cr (VI) is efficiently reduced under *in situ* intestinal incubation and also by upper villus and middle villus cells of rat intestine[57]. The findings indicate that the gut bacteria have marked capacity to cope with the increased load of chromium and may contribute in the protection against chromium toxicity up to a certain extent. In addition, resistance to various antibiotics shown by the resident gut bacteria following chromium ingestion also indicates that use of chromium as nutritional supplement/micronutrient may provide significant protection to the gut microflora, particularly *Lactobacillus*, against some of the commonly used antibiotics. The gut is the natural habitat for a large and dynamic bacterial community. Major functions of the gut microflora include metabolic activities that result in salvage of energy and absorbable nutrients, important trophic effects on intestinal epithelia and on immune structure and function, and protection of the colonized host against invasion by alien microbes[78]. However, altered functions of resident gut microflora following chronic exposure of chromium cannot be ruled out. This, in turn, may adversely affect the body by depriving it of the benefits provided by the microflora that may manifest clinically as various nutritional deficiency syndromes. Thus resident gut microflora plays a very important role in protection against metal toxicity.

Conclusions and future perspectives: Presence of toxic metals in the environment and their ill effects on body, including carcinogenicity is known for a long time. The recent developments have been the understanding of the capacity of microbes to detoxify and eliminate metals. Rapid advances have been made in understanding the biotransformations and the environmental relevance of metal reduction processes by microbes. Now the attention is focussed on the role of gut microflora. However, we still do not know about the precise mechanisms involved and its overall impact. The genomic sequences of main metal-reducing microbes are known, therefore, rapid advances can be expected using the newer techniques and post-genomic and proteomic approaches.

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