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Plasmid DNA Mediated Antibiotic Resistance in Bacteria - A Review

Shanmugasundaram Sridharan and Ramasamy Amsaveni*

Research Department of Biotechnology, Kongunadu Arts and Science College, Coimbatore - 641029, Tamil Nadu, India.

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*Author to whom corresponding should be addressed

Email: amsabiotech@gmail.com

Abstract Environment is contaminated heavily with various pollutants. Air, water and soil are the major natural elements which have gone out of its nature. As a result the element required for life, the food has become toxic which causes related infection. Water pollution, another major global problem which may also be due to contaminated soil and related substances requires continuous evaluation and revision of water resource. It has been suggested that it is the leading worldwide cause of deaths and disease. There are, however a wide variety of soil related infections that also should be considered, particularly in the case of wound, respiratory tract or gastrointestinal infections. Microorganisms, the major population of soil are sensitive to various concentrations of heavy metals; they have mechanisms which enable them to proliferate in the environment by rapidly adapting to that environment by degrading, detoxifying and converting heavy metals into harmless forms through biosorption or enzymatic transformation. Heavy metal resistant microorganisms may be useful as indicators of potential toxicity to other forms of life and are important in studies of genetic transfer in heavy metal resistance mechanism. Thus, individual bacterial strains will develop their capacity to survive under toxicological stress and will be of importance in future bioremediation strategies.

Keywords: *DNA, Antibiotic, Bacteria, Plasmid,*

1. Heavy metals

Heavy metals are the major pollutants which may be exudates of batteries (Pb, Cd, Ni) coatings and paint (Cu, Zn, Cl, TBT) and electrical system (Cu, Pb, Hg) (Ghaderi *et al.*, 2012). Presence of these heavy metals in the biosphere poses severe threat to the environment because of their ability to persist as such for several decades (Matyar, 2012). The main threats to human health from heavy metals are associated with exposure to lead, cadmium, chromium, mercury and arsenic (Nithya *et al.*, 2011). Arsenic and chrome are classified as priority pollutants by the United States Environmental Protection Agency (US EPA) with a carcinogenicity classification A (human carcinogen), while cadmium and lead are classified in the same list with a carcinogenicity classification B (probable human carcinogen) (Pazi, 2011). Persistent toxic metals that settle down on the sediment are a threat to the survival of all organisms and to biodiversity (Haruna *et al.*, 2011). Through bioaccumulation such metals enter in the food chain and bio-magnified.

A high rate of spread of resistant gene has been suspected as the cause of increased antibiotic resistance cases in it. Plasmid carry genes that could be spread by conjugation and transduction while the genome based resistant genes are also spread by replication. It is known that R plasmid-carrying bacteria are transferred to humans, either from food contaminated with animal, bacteria, from other human beings directly or through contaminated food or water. Before treatment, the patients may already harbour multiple drug resistant bacteria at a concentration below the detectability of the methods used, or they may acquire them during the treatment (Manjusha and Sarita, 2011).

The acquisition of genetic information that had been developed in other kinds of living

organisms. By chance, a gene acquisition can provide to the receiving organism a welcome novel function that provides a selective advantage. An example is the acquisition of genetic information for antibiotic resistance by pathogenic bacteria. We see here a sharing in the evolutionary success of other organisms. Of course, horizontal gene transfer can, in other cases, also lead to a selective disadvantage by negative impacts on the functional harmony of the receiving organism (Arber, 2014).

2. Multidrug resistance

Drug resistance among the pathogens was commonly reported in various locations. Over dosage and prolonged use of a particular antibiotic for an infection was the only cause factor for the drug resistance. Multidrug resistance of an organism was reported by several scientists. It is being a great deal for the scientists for overcoming the drug resistance. Nowadays certain compounds like clavulanic acid and sulbactam were given along with the drug to prevent the expression of drug resistance gene or to suppress the pathway of resisting the antibiotic. Many reports were cited for the drug resistance among the Enterobacteriaceae members. Among which NDMBL (New Delhi Metallo-Beta-Lactamase) in *E. coli* was the most recent evidence for the resistance pattern.

Several studies showed that antibiotic resistance characteristics can be transferred to sensitive recipient organisms in the environment and DNA coding for antibiotic resistance may be conjugally transferred between similar microorganisms. Thus an antibiotic-resistant bacterium poses a serious public health problem. Here emerged the importance of detecting such bacteria and such genetic elements and study the possible ways of preventing or at least controlling this phenomenon. The massive works and intensive researches all over the world certify that this point is a real hot issue up till now. Problems associated with the development and spread of antibiotic resistance in the clinic have been increasing since the early 1960s and are currently viewed as a major threat to clinical practice. Plasmid-linked resistance, especially for pathogenic bacteria isolates are still of critical importance. It is generally accepted that the main cause of this problem has been and still is the discharge of antibiotic-resistant bacteria and antibiotics in various amounts in the environment, the widespread inappropriate use and over-prescribing of antibiotics in clinical medicine (Gilbert and McBain, 2003).

Bacterial resistance to antibiotics and heavy metals is an increasing problem in today's society. Resistance to antibiotics is acquired by a change in the gene makeup of bacterium, which can occur by either a gene mutation or by transfer of antibiotic resistance genes between bacteria in the environment. The increasing use of antibiotics in health care, in agriculture and animal husbandry is in turn contributing to the growing problem of antibiotic resistant bacteria (Dhanorkar and Tambekar, 2004). Heavy metals used in industry and in household products are, along with antibiotics creating a selective pressure in the environment that leads to the mutations in microorganisms that will allowed them better survive and multiply (Calomiris *et al*., 1994).

A correlation exists between metal tolerance and antibiotic resistance in bacteria because of the likelihood that resistance genes to both (antibiotics and heavy metals) may be located closely together on the same plasmid in bacteria and are thus more likely to be transferred together in the environment. Bacteria isolated from drinking water showed high resistance to metals and to antibiotic (Kawane and Tambekar, 2004). Antibiotic drug resistance spreads through horizontal gene transfer (HGT) via bacterial conjugation in unicellular populations of bacteria. Consequently, the efficiency of antibiotics is limited and the expected "grace period" of novel antibiotics is typically quite short. One of the mechanisms that allow the accelerated adaptation of bacteria to antibiotics is bacterial conjugation. However, bacterial conjugation is regulated by several biological factors, with one of the most important ones being repression and derepression (Atsmon-Raz *et al*., 2015).

3. Plasmids

Plasmids are extra chromosomal genetic elements that can range in size from several hundred base pairs to several thousand kilobases (Cook *et al*., 2001). Transferable antibiotic resistance is due to plasmids, which is capable of replicating independently. In

many cases, it is circular and double-stranded. Plasmids are widely spread in bacteria and they provide an important extra-dimension to the adaptation of the organisms to hostile changes in its environment, like the presence of antibiotics. Plasmid size varies from 1 to >200 kilobase pairs (kbp) (Russell and Sambrook, 2001). The plasmids hold genetic information that determines antibiotic resistance, replication and transmissibility (Lipps, 2008). Bacterial plasmids (with few exceptions) exist within the cell as circular DNA molecules with a very compact conformation due to super-coiling.

Plasmid-encoded genes represent a pool of mobile DNA that contributes significantly to the adaptation of natural microbial communities. An example of such plasmid-mediated adaptation has been reported previously for microbial populations during periods of pollution stress when the frequency of catabolic plasmids increased by as much as 2- to 10- fold in polluted marine and freshwater ecosystems (Burton *et al*., 1982).

The genetic flexibility of bacteria has contributed to their survival in altered environments, because of their capacity to acquire and transfer resistant genes. Bacteria have developed resistance to all different classes of antibiotics discovered to date. The most frequent type of resistance is acquired and transmitted horizontally either through conjugation, transformation or transduction of plasmid (Frost *et al*., 2005).

4. Causes for drug resistance

One of the main causes of antibiotic drug resistance is antibiotic overuse, abuse and in some cases, misuse due to incorrect diagnosis. A second cause is counterfeit drugs. Antibiotic use in animal husbandry is also creating some drug resistant bacteria, which can be transmitted to humans. Increased globalization could also cause the spread of drug resistance. Finally, hospital settings often give rise to antibiotic resistant bacteria. In addition to chromosomal mutations, a second broad category of drug resistance is due to mobile genetic elements, such as plasmids or transposons, which carry drug resistant genes. Microbial antibiotic resistance and pathogenicity are often associated with HGT, but the scope of HGT extends far beyond disease-causing organisms. In this Review, we describe how HGT has shaped the web of life using examples of HGT among prokaryotes, between prokaryotes and eukaryotes, and even between multicellular eukaryotes (Soucy *et al*., 2015).

5. Horizontal gene transformation - conjugation

Horizontal gene transfer (HGT) is the movement of genetic information between organisms, a process that includes the spread of antibiotic resistance genes among bacteria (except for those from parent to offspring), fueling pathogen evolution (Burmeister, 2015). Horizontal gene transfer enables bacteria to respond and adapt to their environment much more rapidly by acquiring large DNA sequences from another bacterium in a single transfer. The emergence of bacterial isolates that are resistant to an antimicrobial agent represents a continuing ecological battle to achieve a natural host-parasite balance. As new antibiotics are developed and used, resistant strains may develop. In addition, the lack of reports in which physical evidence of the transmissible R plasmids possessed by fish bacteria is noteworthy (Son *et al*., 1996).

A fundamental concept in biology is that heritable material, DNA, is passed from parent to offspring, a process called vertical gene transfer. An alternative mechanism of gene acquisition is through horizontal gene transfer (HGT), which involves movement of genetic material between different species. HGT is well-known in single-celled organisms such as bacteria, but its existence in higher organisms, including animals, is less well established, and is controversial in humans (Crisp *et al*., 2015).

Horizontal gene transfer also known as lateral gene transfer is a process in which an organism transfers genetic material to another organism that is not its offspring. For conjugation, gene transfer mediated by cell-to-cell contact, the donor and recipient strains need to be in the same environment to allow for the creation of the conjugative machinery between the donor and recipient cells. The antibiotic resistance and virulence genes in question need to be transferable, that is they need to exist on mobile genetic elements, segments of DNA encoding proteins important for the mediation of movement of DNA within genomes (Kelly *et al*., 2009). The transported DNA has to avoid the restriction nucleases of

the recipient and these genes need to be recombined efficiently in order for effective future persistence. The recipient has the more active role that promotes the uptake of exogenous DNA in transformation but with conjugative methods, the donor is the more active participant in the DNA transfer process (Thomas and Nielsen, 2005). The majority of bacterial gene transfer events in the environment are through conjugation. There are many types of conjugative events, which all have been shown to occur in natural conditions. Many conjugative plasmids and transposons have been shown to have a broad host range including most Gram-negative bacteria and even some Gram-positive bacteria (Thomas, 1981). Conjugal transfer of resistance plasmids was demonstrated *in vitro* in the 1970s by many groups including: Lakhotia *et al*. (1972), where R factors (plasmids which encode resistance determinants) were transferred between two Gram-negative strains: *E. coli* and *Salmonella*. *In vitro* conjugative transfer between Gram-positive *L. acidophilus* and *L. reuteri* was proven by Vescovo *et al*. (1983).

Plasmids can be classified into what is known as incompatibility (Inc) groups, first introduced by Novick (1987). Plasmids with diverse Inc groups differ in host range of transfer, autonomous replication, pilus structure and size (Droge *et al*., 1998). This is important when considering the dissemination and evolution of plasmids conferring resistances or virulence determinants into strains which may have indigenous plasmids present.

Evidence exists that antibiotic-resistance genes were present in the era before antibiotic therapy was available, and they probably originated from antibiotic-producing bacteria (Medeiros, 1997; Gardner *et al*., 1969). Environmental levels of multiple classes of antimicrobial agents are now so common in soil and water samples that multiple bacterial genera have strains that subsist entirely on antibiotics as their sole carbon source (Dantas *et al*., 2008). These bacteria express remarkably high levels of resistance to a wide array of antibiotic classes.

This plasmid-encoded resistance has been observed for virtually all classes of antibiotics and in a wide variety of Gram-positive and Gram-negative organisms; many antibiotics are no longer effective due to such plasmid-encoded resistance. The systematic removal of these resistance-mediating plasmids from the bacteria would re-sensitize bacteria to standard antibiotics. As such, plasmids offer novel targets that have heretofore been unexploited clinically. This perspective details the role of plasmids in multi-drug resistant bacteria, the mechanisms used by plasmids to control their replication, and the potential for small molecules to disrupt plasmid replication and re-sensitize bacteria to antibiotics (Johna *et al*., 2005).

Heavy metal contamination is widespread. Heavy metals are defined as a group of metals whose atomic density is greater than 5g/cm^3. In nature, there are about 50 heavy metals of special concern because of their toxicological effect to human beings and other living organisms. Many of them, like Zn, Cu, Co, Ni, Mn and Fe have the nutritional characteristics known as essential "trace elements" are necessary for living organisms because at a certain concentration levels, these elements participate some enzyme activities (Bruins *et al*., 2000).

The environmental pollution by heavy metals comes from anthropogenic sources such as smelters, mining, power stations and the application of pesticides containing metal, fertilizer and sewage sludge. By affecting the growth, morphology and biochemical activities, heavy metals influence the microbial population and resulting in decreased biomass as well as diversity. Therefore microbes have developed mechanisms to tolerate the metals either by presence of heavy metals through efflux, complexation or reduction of metal ions or to use them as terminal electron acceptors in anaerobic respiration (Gadd, 1990). Most mechanism reported involves the efflux of metal ions outside the cell and genes for tolerance mechanisms have been found on both chromosomes and plasmids.

6. Conclusion

Bacteria that are resistant to and grow on metals play an important role in the biogeochemical cycling of those metal ions. An increasing problem for the treatment of different infectious diseases is bacterial resistance to antibiotics and other antimicrobial agents. It is thought that a

correlation exists between metal tolerance and antibiotic resistance in bacteria. Thus we need to be more careful of the drastic use of antibiotics in medical practice and also more aware of other antimicrobials that we put into the environment, such as heavy metals. So in this case plasmid isolation and curing was carried out to establish the antibiotic resistance properties with plasmid. The present study reports the characterization of one of the isolate from heavy metal contaminated soil.

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