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Research Article

An Insight into the 2016 Best Medical Award-Winning Breakthrough Microbial and Nanotechnology Based Discovery of Dr. M.S. Reddy's Multiple Mixed Strain Probiotic Therapy, to Successfully Treat the Nosocomial Infections

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Abstract

This review/research article is written to explain in detail the theory behind the success of the widely popular "Multiple Mixed Strain Probiotic Therapy" [1] to prevent or treat the hospital acquired or nosocomial infections due to Clostridium difficile (C. diff), Methicillin Resistant Staphylococcus aureus (MRSA) [1] [2]. A brief explanation of the genesis, history, and definition of Probiotics is also mentioned. In addition, the author's version of the composition of the gastrointestinal flora is presented with special emphasis on the Probiotics. The article also outlines the best way and the form to administer the Probiotics as therapeutic agents. A detailed explanation is given to classify the novel Dr. M.S. Reddy's Multiple Mixed Strain Probiotic Therapy as a Nano and microbial technology therapy.

Keywords: *Nano and microbial technology; Probiotics; Bacteriocins; Dr. M.S. Reddy's Multiple Mixed Strain Probiotic Therapy; Probiotic therapy; hospital acquired infections.*

A recent discovery of Dr. M.S. Reddy's Multiple Mixed Strain Probiotic Therapy to successfully prevent or treat hospital acquired infections, which were infecting over 6,000,000 people and killing over 100,000 people per year, arose a great interest worldwide, in the medical community. This discovery was published in 2016 in the "International Journal of Pharmaceutical Sciences and Nano Technology" [2]. In addition, another follow up publication appeared in 2016 in the same journal, outlining the major principle or causative agent behind the discovery [1].

Before we go into specifics of Dr. M.S. Reddy's discovery, it is worthwhile to understand few basic facts about Probiotics. The latest buzz word in the entire world is "Probiotics". What are Probiotics? They are essentially opposite to antibiotics. Probiotics are the beneficial microorganisms, which are not pathogenic and pyrogenic, which will improve the health of the human being by restoring the healthy gastrointestinal microflora. Human gastrointestinal tract harbors roughly 500 species of bacteria, which in total amounting close to 100 trillion bacteria. Whereas, the total human eukaryotic cells are only roughly 10 trillion. It goes to prove that by sheer number wise, human being is predominantly made of microorganisms, although they are significantly smaller than the eukaryotic cells. Yet, the significance of these intestinal bacteria is not totally understood. However, the eco imbalance of the gastrointestinal flora does result in either specific disease or severe symptoms of discomfort or premature aging.

The next obvious question is, what is the genesis of Probiotics and how did they develop the therapeutic properties? According to the Hindu scripts (Vedas), the fermented milk products have been in existence for over 5000 years, perhaps made with undefined Probiotics and other unknown natural beneficial bacteria. As an evidence, from the time immemorial, the Hindu God "Lord Krishna" has always been depicted consuming the homemade fermented dairy products directly from the earthen pot. The earthen pot will keep the fermented dairy product cool, to eliminate excess acid production. Thus, India should be regarded as the mother country for Probiotics, although such terminology did not exist in the past. Even to date, the practice in India is to make their fermented milk products at home, for their daily consumption. Each house hold maintains their own bacterial culture (which has been passed down from several generations), which we call in the Western world "Starter Culture". In India, most generally, every last course of the meal should end up with significant amount of the highly fermented liquid milk product. It is called Dahi in Northern part of the India, and generally called buttermilk or curd in Southern part of India. The exact bacterial composition of these homemade fermented milk products are not known. Our guess is that they are predominantly wild lactic acid-producing bacteria along with beneficial yeasts. They are prepared using the following procedure. The heat treated (to boiling) and cooled whole milk (to room temperature) is inoculated with previous day's fermented milk

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and then incubated overnight at room temperature for at least 12 to 16 hours. It is consumed as part of the meal next day along with pickle (made of lemon or mango) or fruit. In this case pickle not only acts as condiment but also as prebiotic. It is always consumed as the final course of the meal. Indians treat the mother culture as goddess. They believe that their health cannot be maintained well unless they consume their homemade fermented milk drink or product on a daily basis. Undoubtedly, few thousand years ago they have figured out that consuming the fermented milk products greatly improves their health. In this connection, by taking into account the history of Hindu civilization, Lord Krishna can be considered as the great grandfather of Probiotics [3].

Apparently, although not quite but similar practices might have been followed in several South East Asian countries, Middle East, and parts of Eastern Europe. Whereas, in the West, most of these fermented drinks are manufactured at factory level and then distributed through grocery stores. This practice started only 100 years ago. The defined strain Starter Cultures made by the Starter Culture companies are purchased to manufacture the fermented milk products at the dairy plants, without having to transfer them on a daily basis. The composition of the Starter Cultures is defined and only specific lactic acid bacteria are used as fermentation agents. For example cultures used for manufacturing yogurt are totally different than the once used to manufacture buttermilk. Irrespective of the bacterial composition, the difference between homemade fermented dairy products in India vs. the factory made fermented dairy products in either USA or Europe or Australia is the length of time it takes to consume such product after they are made. In the West, the shelf life of such fermented products is up one to four months and thus are not consumed fresh. In my opinion, the freshly made and consumed (on the same day) fermented milk product will have better therapeutic property than the product consumed one month or four months after it is made, due to the variance in metabolic activity, viability and the growth end products of the beneficial bacteria. Whereas, In India, they are consumed the very same day they are prepared. All in all, these fermented drinks either made at home using undefined lactic acid bacteria or made at factory level using defined cultures are still considered as beneficial bacteria to improve health. Thus the genesis of Probiotics go way back to over 5000 years.

How did these natural lactic acid bacteria develop the potential to inhibit the pathogenic bacteria? Although it was not known over 100 years ago, it is now known that they have intrinsic capacity to produce lactic acid and other metabolic end products (including potent bacteriocins) which not only inhibit pathogenic bacteria but also impart good health to humans [4]. In addition, most of the Probiotic cultures are granted GRAS (generally regarded as safe) status by FDA (Code of Federal Regulations, 2001).

The next obvious question is, what are the benefits of consuming Probiotics? As early as 1907, Dr. Elie Metchnikoff, the Nobel Laurate and Scientist, pointed out that consuming fermented milk products decrease the incidence of intestinal infections. He has also mentioned that consumption of such products extend the life of humans, by reducing the putrefaction in the gastrointestinal tract [5]. However, not much attention has been paid to the teachings of Dr. Metchnikoff. After Dr. Alexander Fleming came up with the discovery of Penicillin, the use of Penicillin was put into practice in the mid-1940's to treat the bacterial infections, which further eliminated the interest of looking into the beneficial bacteria as therapeutic or health promoting agents. However, the entire scientific community did not realize that the pathogenic bacteria eventually will develop resistance to such antibiotics over the period of time and thus develop into superbugs. In fact it did happen. The antibiotics not only killed the pathogenic bacteria but also killed the natural nonpathogenic beneficial bacteria (Probiotics). Surprisingly, until the mid-1960's nothing was heard of any therapies involving microorganisms, despite the fact Nobel Laureate Dr. Elie Metchnikoff pointed out the possible benefits of consuming beneficial bacteria to improve the gastrointestinal health [6]. In this connection we can call Dr. Metchnikoff as grandfather of Probiotics [6]. To eliminate the redundancy, we strongly recommend the articles written by Reddy and Reddy regarding the detailed benefits of Probiotics to improve the health [3], [7], [8], [9], and [2].

The term Probiotic was only coined in 1965 by Lilly and Stillwell, in a different context than using them as the health promoters [10]. In the year 1974 Parker described Probiotics as "organisms and substances which contribute to intestinal microbial balance" [11]. In 1989, Fuller published an article in the Journal of Applied Bacteriology which redefined Probiotics as "live microbial supplements which beneficially affects the host by improving its microbial balance [12]. Later in 1998 Salminen et. al. defined Probiotics as "foods containing live bacteria which are beneficial to health" [13]. In the year 2001, United Nations Food and Agriculture Organization and the World Health Organization (FAO/WHO) in conjunction with the Canadian Research and Development Centre for Probiotics, came up with the following consensus definition for Probiotics, which is as follows: "Probiotics are any live microorganisms which when administered in adequate amounts confer a health benefit on host" [14]. This is the latest (published in 2007) and widely used definition and it encompasses all applications of live microorganisms, not just those for the gastrointestinal health benefits [6].

In this connection, credit should go to Reddy et. al. [15] who have for the first time in the history in the year 2000 came up with a novel patented idea of introducing Probiotics as the integral and functional part of the therapeutic drugs, to enhance the drug efficiency, without any side effects. They have discovered that Probiotics when used as part of active ingredients of the drug, significantly improved the therapeutic value of the drugs (allopathic, ayurvedic, herbal, and periodontal). This discovery was considered a breakthrough and was granted several patents all over the world and brought immense recognition to Probiotics as viable therapeutic agents like pharmaceutical drugs, although they are all-natural and biological in nature.

Today we are into another serious problem because of the mutated multiple antibiotic resistant pathogenic bacteria which do not respond to antibiotic therapy and thus killing millions of people. One such example which is bothering the entire medical community, all over the world, is hospital acquired infections due to Clostridium difficile (C. diff), Methicillin Resistant Staphylococcus aureus (MRSA), Klebsiella pneumoniae, and the Carbopenium resistant pathogenic strains belonging to Enterobacteriaceae. The hospital and health care providing centers became the breeding and harboring grounds for these hospital acquired or nosocomial infections. For more details we strongly refer the reader to 2016 articles of Reddy and Reddy [1]. According to the United States Center for Disease Control and prevention some of these hospital acquired infections are spreading into the mainstream causing kidney diseases, pulmonary diseases and other complications and in some cases with a scary 50% mortality rate.

How can we control these nosocomial infections, if the antibiotics are not working? This is where the 2016 discovery of Dr. M.S. Reddy's Multiple Mixed Strain Probiotic Therapy comes into play [1] and [2]. The concept here is to use the therapeutic all natural biological Probiotic cultures to inhibit the multiple antibiotic resistant pathogenic bacteria through competency. The next obvious question, what is and how does Dr. M.S. Reddy's Multiple Mixed Strain Probiotics inhibit or kill the nosocomial infection causing multiple antibiotic resistant pathogenic bacteria? More specifically, what is Dr. M.S. Reddy's "Multiple Mixed Strain Probiotic Therapy"? Dr. M.S. Reddy's Multiple Mixed Strain Probiotic Therapy is defined as a biologically based therapy comprising several naturally antibiotic and antimicrobial agent resistant strains of Probiotics belonging to

different genera and species, along with their growth end products and bacteriocins, to treat the nosocomial or hospital acquired infections [1] and [2]. The success of Dr. M.S. Reddy Multiple Mixed Strain Probiotic Therapy is predominantly due to the preformed all natural Nano particles produced by the Probiotic strains in addition to the live Probiotic cultures.

The size of individual Probiotic bacterial cell will be in the range of 100 to 200 nanometers. However when they are grown in a specific nutrient media, these Probiotic cultures produce exo-enzymes which will significantly reduce the size of the nutrients (including mineral complexes) to smaller than 100 nanometers, so that they can be transported into the bacterial cell. During this process, due to the enzyme hydrolysis, significant amount of active Nano particles will be generated (10 to 90 nanometers size) and majority of them will be left in the growth medium. In addition, the Probiotic cultures by utilizing the hydrolyzed Nano nutrients, will further synthesize (intracellularly) several other all natural Nano compounds which will be excreted into the growth medium. Some of these Probiotic bacteria synthesized all natural Nano particles are bacteriocins.

We can safely assume that these synthesized all natural Nano particles should be less than 50 nanometers in size because the bacteria synthesizing them is only 100 to 150 nanometers in size (to facilitate transport of such particles, out of the cell, through membrane transport system). Consequently the entire growth medium, after fermentation by Probiotic cultures will have significant amount of charged all natural Nano particles (10 to 100 nanometers in size). In addition, some of the organic acids produced by the Probiotic bacteria will react and decrease the size of the metal salts (also solubilize and ionize them) present in the growth medium to less than 100 nanometers to transport such metal ions into the cell through membranes. The left over soluble Nano metal ions, which were not consumed by bacteria, will be present in the culture medium. Thus the entire growth medium, after the growth of the Probiotics, will have several all natural Nano particles with their sizes ranging from 10 to 100 nanometers. These Nano particles are small, highly charged, have larger surface area, and act as potent inhibitors against several pathogenic bacteria. In order to classify any technology as a Nanotechnology, the particle sizes should be less than 100 nanometers. According to this definition, the individual Probiotic bacterial cell with a size of 100 to 200 nanometer cannot be considered or categorized as Nanotechnology product. However, the majority of the bacterial growth end products (either left in the growth medium after Probiotic bacterial enzyme hydrolysis or synthesized and excreted into the growth medium by Probiotics) should qualify as all natural Nanotechnology products.

Dr. M.S. Reddy's Multiple Mixed Strain Probiotic Therapy is a combination of all natural Nano and microbial technology. It is the most marvelous concept in that 100 to 150 nanometer size bacteria can be constantly producing smaller Nano particles. It has been proven by Dr. M.S. Reddy that the hospital acquired infections due to C. diff and MRSA responded better when Multiple Mixed Strain Probiotics were administered along with their growth end products including the bacteriocins (Nano particles size of less than 100 nanometers). This explains why Dr. M.S. Reddy's Multiple Mixed Strain Probiotic Therapy is highly successful to treat or prevent the nosocomial infections because of the presence of abnormal number of Nano particles of size smaller than 100 nanometers along with the active Probiotic bacteria [1,2]. Thus, Dr. M.S. Reddy's Multiple Mixed Strain Probiotics Therapy encompassing several strains of Probiotics belonging to different genera and species along with their all natural Nano particles (size <100 nanometers), growth end products including bacteriocins, frozen using liquid nitrogen cryogenic technology, should technically qualify to be called Nanotechnology therapy.

In the human gastrointestinal tract there are over 500 species of bacteria with a total population of 100 trillion organisms. The next question is how many out of these 100 trillion bacteria are Probiotics? It is impossible to predict because of variance of microflora in each individual, which in turn depends on their diet, health, and life style. If we have to take a scientific guess, in a healthy gut perhaps 15 to 25% of them may be Probiotics bacteria, 60 to 85% bacteria may be normal commensals which are nonpathogenic, 2% or less may be opportunistic and yet subdued pathogens, and perhaps less than 0.01% may be subdued virulent pathogens. In other words (according to our assumption) 15 to 25 trillion bacteria are truly Probiotics or the organisms which confer the health by maintaining the proper eco balance in the human gastrointestinal tract.

In our opinion, if the Probiotic population is decreased by 75 to 100%, the opportunistic and subdued pathogens will dominate and cause the gastrointestinal disease. Some of the pathogenic Clostridium and Staphylococcus species stay as dormant flora as long as the Probiotic population is at their normalcy. When once the Probiotic population is killed or reduced significantly due to administration of antibiotics etc., the pathogenic flora will dominate and cause the GI tract infections. In our opinion, in order for pathogens to become dominant flora, certain Probiotics which were protecting the gut has to come down at least by 90 to 95% i.e. from 15 to 25 trillion down to 0.75 to 1.25 trillion. Also when once the Probiotics are reduced or killed, the receptor sites once occupied by Probiotics will be occupied by the pathogens and their toxins.

In order to replenish the Probiotic bacteria (which were killed by antibiotics), we may have to supplement the Probiotics. Assuming that 14.25 to 23.75 (average 19 trillion) trillion Probiotic bacteria were killed, we may have to supplement at least 190 trillion Probiotic bacteria, since a maximum of 10% or less of such bacteria will implant. Consequently, just giving a lyophilized Probiotic culture at a concentration of 100 billion Probiotic bacteria will only implant 10 billion organisms or perhaps even less. In other words we were able to implant less than 0.1% of the total Probiotic population. According to several reports, a freeze dried preparation, although it is reported as having 100 billion organisms/ per gram at the time of inception, in reality they will not have more than 10 billion organisms/gram at the time of consumption. That too the organisms in the lyophilized preparations are not in an excellent physiological condition to out-compete the pathogens. In other words, the use of freeze dried Probiotic cultures as therapeutic agents is not very effective. Reddy and Reddy in 2016, proved that freeze dried Probiotics are not as effective as liquid nitrogen frozen liquid cultures to treat the C. diff and MRSA infections both in vitro and under the practical hospital conditions [1].

According to Dr. M.S. Reddy's Multiple Mixed Strain Probiotic Therapy, a daily dose of 330 grams of the liquid nitrogen frozen Multiple Mixed Strain Probiotic culture should be administered with a total concentration of approximately 3 trillion Probiotics per dose, along with their growth end products and bacteriocins (Nano particles), to successfully treat the C. diff infection [1]. The experiments clearly proved that the Probiotics even at a concentration of 15 trillion per dose could not cure C. diff infection when administered without their growth end products and the bacteriocins (Nano particles). In other words, even a low bacterial concentration of liquid nitrogen frozen Multiple Mixed Strain Probiotics were more effective to treat C. diff infection, when administered along with their growth end products including bacteriocins, compared to high bacterial concentration lyophilized Probiotics. Apparently, the growth end products and bacteriocins start the initial process of inhibiting or killing the pathogenic organisms in the gastrointestinal tract and thus allowing the live Probiotics to implant and further inhibit the pathogens, as a secondary process. Reddy and Reddy have determined that freeze dried cultures have 50% less efficiency in retarding the pathogens, even though they have higher cell numbers, because of the lack of the

growth end products and bacteriocins [1].

Considering the above scenario, consuming a mega dose of liquid nitrogen frozen mixed Probiotic culture once a week (if possible twice or three times a week) is much more effective than consuming freeze dried capsules daily, as a preventive measure. Of course, the dose to be used as therapeutic agent is totally different. We have to realize that convenience alone (freeze dried as opposed to frozen liquid cultures) is not the answer for efficacy and function of the Probiotics. The technology of manufacturing the Probiotics has to be improved to maximize the production of their beneficial health promoting end products of fermentation, along with the improved bacterial cell numbers and viability.

In summary, Dr. Reddy's Multiple Mixed Strain Probiotic Therapy points out that not all Probiotics are same and the composition and method of preparation of Probiotic cultures dictate their efficacy as preventative or therapeutic agents. In addition, considering the diversity of microflora in the gastrointestinal tract, administering a pretested naturally antibiotic resistant Multiple Mixed Strain Probiotics, along with their growth end products including Nano particle bacteriocins, frozen in liquid nitrogen is the best way to maximize the preventative or curative effect of these Probiotics on both the nosocomial infections as well as other gastrointestinal infections [1,2].

Special Editorial Comment:

The editor and reviewer of this article Dr. Antonio Siniscalchi, made the following comments on the discovery of Dr. M.S. Reddy's Multiple Mixed Strain Probiotic Therapy: "This research article is very well presented. I have also gone through the presented findings and cited publications of Dr. M.S. Reddy with reference to his discovery of Multiple Mixed Strain Probiotic Therapy. In my professional opinion, Dr. M.S. Reddy's discovery is a breakthrough in treating hospital acquired infections. Probiotic bacteria offer a number of potential benefits when administered in sufficient amounts that inpart includes reducing the number of harmful organisms in the intestine, producing antimicrobial substances and stimulating the body's immune response. Dr. Reddy's article was thorough and clear to point out the precise amount of dosage of Probiotics and the results and discussion comply with the methods followed. The methods followed and reported proves that physicians and surgeons, across the globe, can safely and confidently practice Dr. M.S. Reddy's discovery. Publishing this manuscript will definitely help the researchers/clinicians across the globe. Please publish the paper in the present format without any modifications, and I am very happy and privileged to review this article, and even interested in evaluating the further proceedings on the paper."

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