Development of Multiple Mixed Strain Probiotics for “Probiotic Therapy” under Clinical Conditions, to Prevent or Cure the Deadly Hospital Acquired Infections due to *Clostridium difficile* (C. diff.) and *Methicillin Resistant Staphylococcus aureus* (MRSA)\(^*\)

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**ABSTRACT**

This research article outlines the development and use of multiple mixed strain Probiotics, selected on the basis of their natural antibiotic and antimicrobial resistance to antibiotics such as vancomycin, bacitracin, and sulfonamides, in an attempt to successfully override the hospital associated infections due to *Clostridium difficile* (C. diff) and *Methicillin Resistant Staphylococcus aureus* (MRSA). Suitable procedures were developed to study the antibiotic resistance or sensitivity of the Probiotics and other gastrointestinal tract associated flora. The associative growth relationships of the mixed strain Probiotics were determined prior to pairing them in terms of their compatibility, using the differential plating techniques and phage typing. The specific phages infecting several Probiotics have been isolated and the techniques were developed to study the strain dominance among the stains of the same species of Probiotics using the bacteriophages. The effect of individual strains of the multiple mixed strain Probiotics on the inhibition of *C. diff* and *MRSA* has been evaluated and compared to the level of inhibition if all the Probiotic strains combined as mixed cultures. The synergetic effects of mixed Probiotic cultures in combination with the vancomycin, bacitracin and sulfamethoxazole to inhibit the growth of *C. diff* and *MRSA* was evaluated. Using the results of associative growth studies, antibiotic resistance and sensitivities, phage patterns, a prophylactic and curative or therapeutic blend of the multiple mixed strain Probiotics were developed (using cryogenic technology) and tested using community based clinical trials in the hospital atmosphere.

**KEYWORDS:** Probiotics; bacteriocins; bacteriophage; antibiotics; strain dominance; cryogenics; *MRSA*; *C. diff*; probiotic therapy; multiple mixed strain probiotics; hospital acquired infections; probacteriocin; prebiotics.

**Introduction**

Over 100,000 innocent people die every year due to hospital acquired or associated (nosocomial) infections due to antibiotic resistant bacteria, which are primarily, Methicillin Resistant *Staphylococcus aureus* (MRSA) and *Clostridium difficile* (C. diff) (Reddy and Reddy 2008). Perhaps over four or five decades ago, the prevalence of these infections was very minimal. The overuse and abuse of both narrow spectrum and broad spectrum antibiotics created these antibiotic resistant mutants which turned out like lethal killers. The lack of proper sanitation or negligence or due to continuous influx of the bedridden or hospitalized patients in the hospital atmosphere made hospitals and clinics as favorite sites for the prevalence and survival of these pathogenic bacteria. Most of the time, people are hospitalized due to sickness, which directly or indirectly reduce the patient’s immune system. Also the medications and side effects of the allopathic medicines weaken the immune system of the patient. Thus hospitalized patients are highly susceptible to these deadly hospital associated infections. In addition, the naturally immune compromised individuals will contact these infections at a significantly higher rate. It became a serious subject all over the world, both in the medical community as well as the World Health Organization (WHO). World Health Organization is in the process of discouraging or

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