

INTRODUCTION

Diarrheal diseases are one of the major health problems persisting in the developing countries and can be life threatening especially for infants and young children. Myriad of microbes are responsible for causing acute diarrhea/dysentery. *Shigella dysenteriae* is one of the most virulent bacterial pathogens causing contagious diarrhea that can be fatal especially in infants [World Health Organization (WHO), 2005a]. *S. dysenteriae* is a Gram-negative facultative anaerobe belonging to the family, enterobacteriaceae and is responsible for causing intestinal problem known as 'shigellosis'. *Shigella* infection is a major public health problem in developing countries where sanitation is poor. Worldwide, the incidence of shigellosis is estimated to be 164.7 million cases per year, of which more than 99% are in developing countries (WHO, 2005a; Bhattacharya *et al.*, 2012). Shigellosis accounts for over one million deaths on annual basis. Infection by *Shigella* is not uncommon amongst Indian population, especially children (Taneja, 2007) and many deaths by *Shigella* have been reported in India (Thapa *et al.*, 1998; Taneja, 2007). The control of *Shigella* infections is mediated by the use of antibiotics as there is no effective vaccine at the moment. Unfortunately, the excessive use of antibiotics has resulted in the emergence of drug resistant *Shigella* isolates (Bhattacharya *et al.*, 2012). A concerted effort is going on to reduce the incidence of development of drug resistance in *Shigella* and has forced the scientists to develop new and natural alternatives to prevent and treat diseases. One approach, to reduce the development of drug resistance in *Shigella* is the use of 'probiotics' in conjunction with normal drug therapy.

Humans are getting exposed to numerous gut pathogens and treatment for the same has been limited due to emerging drug resistance among pathogenic species. To have a healthy intestine, the balance of the bacterial flora residing inside the gut must be maintained, but this is difficult as the lifestyle of human changes with passage of time. Lots of factors may change the microbial balance away from potentially beneficial or health promoting bacteria like *Lactobacillus* and *Bifidobacterium* to potentially harmful or pathogenic microorganisms. This shift makes the host more susceptible to illnesses. In such condition, the prevalence of beneficial bacteria must be supported. Alteration in dietary patterns focusing on the use of probiotics as one of the means of bio-ecological control is on the forefront.

Use of probiotics is established since traditional times in form of cheese, curd, yogurt and fermented foods which were part of regular diet. The scientific interest in the area of probiotics came to light with the publication of the book 'The Prolongation of Life', by Nobel

Laureate, Elie Metchnikoff, in the early 20th century. It was well documented that consumption of lactic acid bacteria, mediated fermented milk products by Bulgarian population, led to their longevity and improved health conditions. This remarkable observation of Metchnikoff contributed to the emergence of probiotics.

Probiotics are live microorganisms which when administered in adequate amounts confer a health benefit on the host (WHO, 2002). The word 'probiotic' comes from Greek language 'probios' which means 'for life' opposed to 'antibiotics' which means 'against life'. Therefore, these bacteria affect the gut microflora positively and decrease the microbial toxicity in human gut (Cakir, 2003; Carlet, 2012). Thus, probiotic is a live microbial supplement which affects host's health positively by improving its intestinal microbial balance. Probiotics have been used since long times in food ingredients for human and animals without any side effects. Also, probiotics are acceptable because they are naturally present in the intestinal tract of healthy humans and in foods (Cakir, 2003; Gerritsen *et al.*, 2011). Use of probiotics, helps to protect the host from various intestinal diseases and disorders due to increase in the number of beneficial bacteria that make the intestinal microbial balance steady (Fooks and Gibson, 2002; Gerritsen *et al.*, 2011). Probiotics therapy has been shown to be quite successful either in eradication of microbial infections or reducing their severity (Rolfe *et al.*, 2000; Rishi *et al.*, 2009, 2011) or the use of antibiotics (Pazhani *et al.*, 2004, 2008; Rishi *et al.*, 2009). Lactobacilli probiotics are being considered as strong candidates for prophylactic and therapeutic control of microbial infections either alone or in conjugation with antibiotic treatment (Gopal *et al.*, 2001; Denli *et al.*, 2003; Hempel *et al.*, 2012). Prospective probiotics must tolerate lysozyme mediated proteolysis in mouth, harsh acidic conditions prevailing in stomach, tolerance against bile salts secretion and ability to adhere and colonize gastrointestinal tract (GIT) (Puniya *et al.*, 2012; Pithva *et al.*, 2014). Antagonism against pathogenic microbial species is one of the most crucial properties of probiotic strain (Puniya *et al.*, 2012). Along with this, antibiotic susceptibility pattern of potential probiotic strains should be well studied to minimize risk of antibiotic resistant gene transfer to other pathogenic or unrelated bacteria residing in human GIT (Gueimonde *et al.*, 2013). Hence, microorganisms fulfilling all the desired attributes can be explored as probiotic to combat number of microbial infections. The present study was designed keeping in mind, limited reports on role of probiotics in controlling *Shigella* mediated infections (Moorthy *et al.*, 2007; Nawaz *et al.*, 2011). Based on the above fact, application of probiotic cocktail for prevention or reduction of shigellosis caused by *S. dysenteriae* was undertaken. There are

limited number of reports explaining mechanism behind lactobacilli mediated killing of *Shigella* in macrophages and intestinal epithelial cells (Moorthy *et al.*, 2010). In the current study, novel *Lactobacillus* strains of human origin, possessing probiotic attributes and antimicrobial activity against common pathogens have been investigated and exhaustive work was carried out to study probiotic effects of lactobacilli to combat *S. dysenteriae* effects at microbiological and immunological level.

Keeping above in mind, the present work entitled “**Anti-*Shigella* activity of probiotic lactobacilli isolated from human infants**” has been undertaken. The objectives of this research work were as following:

- **Isolation and screening of lactobacilli strains for potential probiotic attributes**
 - ▶ Isolation and partial characterization of lactobacilli strains
 - ▶ Screening of the lactobacilli isolates for anti-microbial especially anti-*Shigella* activity
 - ▶ Screening of the lactobacilli isolates for potential probiotic attributes
 - ▶ Complete identification of selected lactobacilli isolates
- ***Ex vivo* studies on rat peritoneal macrophages to check efficacy of probiotics against *S. dysenteriae***
 - ▶ Phagocytic activity
 - ▶ Extent of lipid peroxidation
 - ▶ Activity of superoxide dismutase and levels of glutathione
- **Human colonic cell line (Caco-2 or HT29) studies:**
 - ▶ Effect of various treatments of lactobacilli on its ability to inhibit adherence and/or internalization of *S. dysenteriae* to cell line
 - ▶ Expression analysis of immunomodulatory factors
- **Study of anti-*Shigella* properties (lactic acid, hydrogen peroxide and bacteriocin) of selected probiotic lactobacilli**
 - Determination of minimum inhibitory concentration of anti-*Shigella* factors
 - Time kill assay