A Study of Probiotic *Bacillus subtilis* HU58 for the Management of Antibiotic-Associated Diarrhoea in Adults

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Abstract

Aim: **To evaluate the effectiveness of a novel probiotic *Bacillus subtilis* HU58 in an open-labelled placebo-controlled trial in a group of 60 patients with Antibiotic Associated Diarrhoea (AAD).**

Materials and Methods: **The study was conducted in 60 patients suffering from AAD. Patients received either Probiotic *Bacillus subtilis* HU58 (2 X 10^9 CFU/Cap) or placebo for 7 days and followed up to 15th day. Stool consistency was recorded during the baseline, 3rd day, 7th day and 15th day and analyzed according to the Bristol stool chart. The trial was approved by an Independent Ethics Committee and registered with CTRI. Before enrolment, an informed written consent was obtained from the patients.**

Results: **Stool consistency as assessed according to the Bristol stool chart had decreased in the probiotic treated group ($p<0.0001$) as compared to the placebo group ($p=0.8003$). At baseline the stool consistency measurements according to the Bristol stool chart in both groups (probiotic group and placebo group) were found to be 7. The stool frequency had reduced from 7-8 stools per day to 1-2 stools per day. Significant improvement was observed in 16 patients (scale 4), mild improvement observed in 6 patients (scale 5) and no improvement was observed in 8 patients (scale 6) as observed till the 15th day. There was no reduction of score in the placebo group (scale 7) till the 15th day. Also the stool frequency in this group had just reduced from 7-8 stools to 3-4 stools per day.**

Conclusions: **This study had shown that Probiotic *Bacillus subtilis* HU58 at a dose of 2 X 10^9 CFU/Cap once a day for 7 days was well tolerated and safe and compared to placebo the probiotic group significantly showed a reduced incidence of AAD.**

Keywords: **Probiotic *Bacillus subtilis* HU58, Antibiotic Associated Diarrhoea (AAD), Colony Forming Units (CFU), Bristol Stool Chart, Stool consistency.**

Conflict/s of Interest: **Dr. Dilip Mehta, Dr. Anselm de Souza and Dr. Shashank Jadhav are CEO, Managing Director and Med. Director from Synergia Life Sciences Pvt. Ltd. respectively.**

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Introduction

Gastrointestinal disturbances are frequent side effects of antibiotics especially broad-spectrum ones like Vancomycin, Amikacin, Gentamicin and the third generation β-lactams. The mechanisms for this occurrence is not yet fully understood but it could be due to the fact that antibiotics do affect the viability and function of normal bowel flora; the loss of this normal flora could cause an overgrowth of pathogenic species such as Staphylococcus, Candida, Enterobacteriaceae, Klebsiella, and Clostridium or the antibiotics could also directly affect the intestinal mucosa and disturb the bowel movement of the gastrointestinal tract. These disturbances are commonly termed as antibiotic-associated diarrhoea (AAD) [1].

Depending upon the type of antibiotic, population, rates of antibiotic-associated diarrhoea are seen in between 5-39% and 5-30% of patients antibiotic-associated diarrhoea is seen during initial therapy[2,3]. The estimated antibiotic-associated diarrhoea in hospitalized patients is around 10-15% and the infection rates for C. difficile due to the use of antibiotics are reported to be around 10% after 2 weeks of hospitalization and could reach 50% over 4 weeks[4].

In children the prevalence of antibiotic-associated diarrhoea (AAD) is around 11%[5]. In a study involving 650 children aged 1 month to 15.4 years treated with oral antibiotics the incidence of AAD was 11%[6]. In gastrointestinal diseases and diarrhoea associated with antibiotics, probiotics could be beneficial[7].

Probiotics are often proposed along with antibiotic treatment with the belief that the ingestion of 'healthy' bacteria will reduce episodes of AAD. Many trials have reported probiotic preparations in various settings and with different outcomes. However, comparison between studies is challenging due to the variables like strains of bacteria used, single or combination strains, mode of administration of the probiotic, dose of the probiotic, etc[8]. Many reviews have been reported that probiotics reduce AAD[9,10].

Materials and Methods

Study Design

This study was an open-labeled, placebo-controlled study for comparison of the efficacy of the Probiotic *Bacillus subtilis* HU58 with placebo in AAD patients. The study was conducted at Kokan Hospital, Mumbai after approval from Inter System Biomedica Ethics Committee (ISBEC/NR-35/KM-VM/2017), Vile Parle, Mumbai and registered with Clinical Trial Registry of India (CTRI/2018/01/011186).

Inclusion criteria

- Male and female patients between 18 to 65 years
- Patients having stable ECG
- Patients having AAD
- Patients willing to give informed consent

Exclusion criteria

- Sero-positive patients for HIV, HBsAg, and HCV
- Pregnant females
- Patients who were on other probiotics or prebiotics,
- Patients who are addicted to tobacco and alcohol
- Patients who are on any medication other than the prescribed oral antibiotics.

Primary outcomes

To evaluate the role of Probiotic- *Bacillus subtilis* HU58 in humans with antibiotic-associated diarrhoea in comparison with placebo.

Secondary outcomes

Safety and tolerability of Probiotic capsules consumed orally by the subjects and clinical variables based on the laboratory parameters for clinical biochemistry and organ function tests.

Study Procedure

A total of 60 patients who met the inclusion criteria were selected for the study after a proper history taking, examination and investigations as per the criteria of choice mentioned in the protocol.

At the first visit to the hospital baseline blood investigations which included complete blood counts with ESR, PT-INR, serum creatinine, homocysteine, liver function tests, routine urine analysis, routine stool analysis, ECG, HIV, HCV, and HBsAg were done. On the 3rd day complete blood counts with ESR, PT-INR, serum creatinine, homocysteine, routine urine analysis, routine stool analysis, liver function tests were done. All these were repeated on the seventh 7th day along with an ECG. Follow up was done for patients on 3rd day, 7th day and 15th day. At each visit to the clinic, a detailed physical (general and systemic) examination was done. A predesigned case record form (approved by the Ethics Committee) which included a page of adverse events was used.

Complete blood counts were done by the PC 210 ERMA Blood Cell Counter. ESR was done by the Wintrobe method. Liver function test were done by Biochemical method and homocysteine estimation were done by RIA/ ELISA/ CLIA method.

Probiotic *Bacillus subtilis* HU58 (2 X 10^9 CFU/Cap) and identical placebo in the form of capsule (7 capsules

per bottle) were supplied by Synergia Life Sciences Pvt. Ltd. At the time of enrollment, capsules were supplied in bottles to patients. The patient ingested one capsule containing 2 X 10^9 CFU after meal for 7 days. The compliance was judged by counting the capsules in the bottle which was brought back at the follow-up visits. The patient was said to be compliant if he /she had consumed a minimum of 80% of the total dispensed capsules.

Statistical Analysis

Data of the 60 patients were analyzed by using students unpaired t-test method. The unpaired t-test for probiotic *Bacillus subtilis* HU58 group was found to be extremely statistically significant (p value< 0.0001) compared to the placebo group (p=0.8003). Table 3 shows the statistical analysis of both the Probiotic *Bacillus subtilis* HU58 group and the placebo group.

Table 3: Statistical Analysis

Diagnosis	Antibiotic associated diarrhoea			
Symptoms	Diarrhoea			
Treatment	Probiotic *Bacillus subtilis* HU58		Placebo	
N	30		30	
	Baseline	15th day	Baseline	15th day
Bristol stool chart	7	4	7	7
Mean	6.57	4.57	6.50	6.53
SD	0.50	1.17	0.51	0.51
SEM	0.09	0.21	0.09	0.09
T	8.6293		0.2541	
Df	58		58	
standard error of difference	0.232		0.131	
two-tailed P value	<0.0001		0.8003	
Confidence interval	95%		95%	
95% confidence interval of this difference from	1.54 to 2.46		-0.30-0.23	

Table 1: Gender of patients consuming Probiotic *Bacillus subtilis* HU58 and Placebo

Probiotic *Bacillus subtilis* HU58 group		Placebo group	
Male	Female	Male	Female
16	14	18	12

Table 2: Comparison of Bristol scale scores from baseline to end of 15th day

	Antibiotic associated diarrhoea	
	Probiotic *Bacillus subtilis* HU58	Placebo
No. of subjects	30	30
Baseline	7 (0.50)	7 (0.51)
End of 3rd day	6 (0.71)	6 (0.50)
End of 7th day	4 (1.19)	7 (0.51)
End of 15th day	4 (1.17)	7 (0.51)

Values are expressed in Mean (SD); SD= Standard Deviation

Results

Sixty participants were recruited for the study and all completed the study. A written informed consent (approved by the ethics committee) was taken from all the patients. Baseline characteristics were similar in both groups but there were more males in the probiotic group compared to the placebo group. Table 1 shows the gender of patients who consumed Probiotic *Bacillus subtilis* HU58 and placebo.

The Bristol stool scale (Fig 1) which classifies the form of human stool into seven categories which was used to measure the consistency of stool during the study[11]. According to the Bristol stool chart the seven types of stool are type 1-2 (constipated), type 3-5 (normal), type 6-7 (loose stool-diarrhoea). Bristol stool scales were further placed into a binary categorical group consisting of Normal stool (Bristol 3, 4 and 5) and Non-normal stool (Bristol 1, 2 and 6).

The Bristol stool scale at baseline for both the groups i.e., probiotic and placebo were at scale 7. In the probiotic group, 16 patients out of 30 had improvement in stool consistency (scale 5) by the third day. By the end of the 7th day, more improvement in stool consistency was observed (scale 4). During the follow up till the 15th day the stool consistency remained similar to the 7th day (scale 4). Patients were feeling better with the therapy. Mild improvement in stool consistency was seen in further 6 patients by the 3rd day (scale 6) and slightly improved by 15th day to scale 5. Eight patients had no improvement in stool consistency (scale 6). The stool frequency on average reduced from 7-8 per day to 1-2 stools per day. In the placebo group, till the end of the 15th day the scale was found to be same as baseline

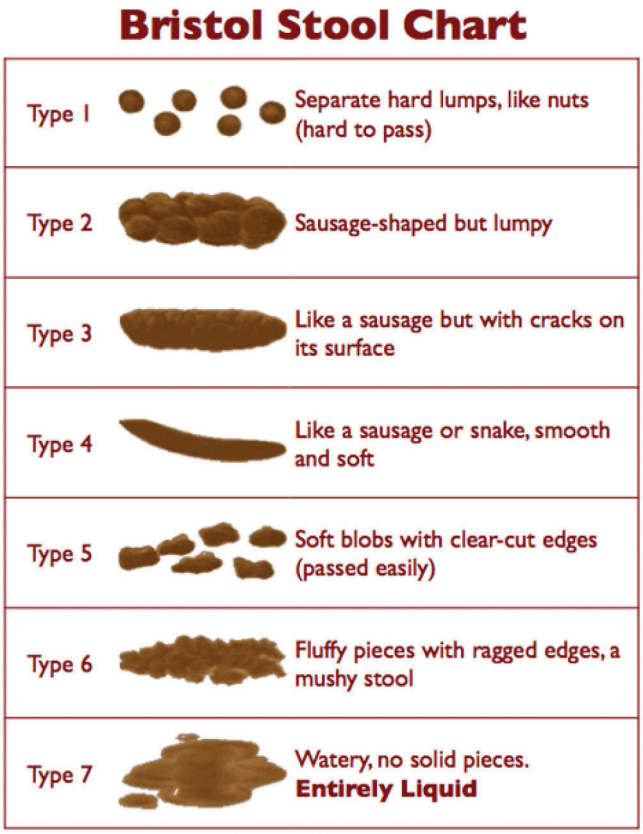

Figure 1: Bristol stool chart [11]

which was at 7. Though the average stool frequency reduced from 7-8 stools per day to 3-4 stools per day, there was no reduction found in the consistency of the stool. Patients showed no improvement with the therapy. Table 2 shows the details about average stool consistency. Figure 2 and 3 shows the graphical representation of the stool consistency in probiotic and placebo group.

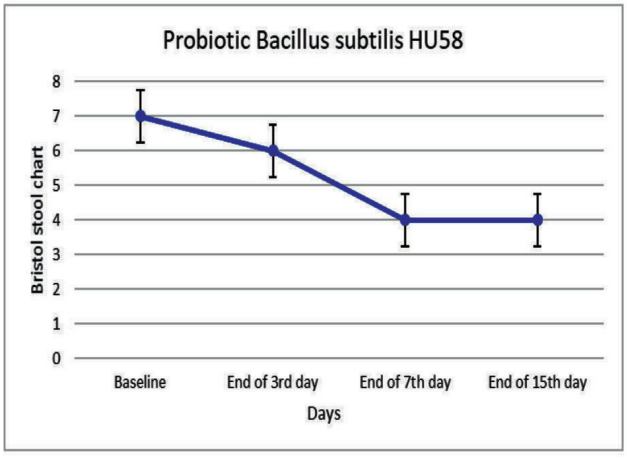

Figure 2: Stool consistency in *Bacillus subtilis* HU58 group

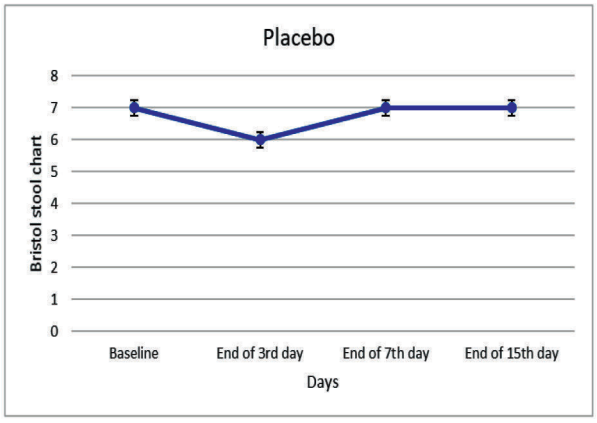

Figure 3: Stool consistency in placebo group

Discussion

Bacillus subtilis strains produce bacteriocins, subtilin and subtilosin[12] and have also been extensively studied at genetic and physiological levels[13]. These strains have been shown to suppress traveler's diarrhoea caused by the pathogen Citrobacter rodentium in a murine model[14] and improve clinical, microbiologic and immunologic efficacy in acute infectious diarrhoea in young children[12]. In poultry, *Bacillus subtilis* has been shown to suppress pathogenic infections caused by Salmonella enterica,[15] Clostridium perfringens[15] and Escherichia coli[16]. An in-vitro study has shown potential for use against Helicobacter pylori[17].

The HU58 strain of *Bacillus subtilis*, isolated from healthy human volunteers has been studied extensively in detail by Prof. Simon Cutting and his group at the Royal Holloway College University- London and has been proven to be more stable in acidic stomach environment. It can grow and sporulate in the anaerobic GI tract with high sporulation efficiency, form biofilms that enhance gut colonization and produces surfactants that enhance the gut adhesion[18,19].

Oral supplementation of probiotics provides a useful supply of beneficial bacteria for gut health, aiding nutrition and potentially stimulating the immune system. A recent open-labeled study was conducted by Dound YA *et al* in which 18 healthy subjects were administered probiotic *Bacillus subtilis* HU58 capsules (2 billion CFU/Cap) once a day orally for 8 weeks. At the end of 8th week, it was observed that there was a reduction of 45% in IL-6 levels and 55% in TNF-α levels[20].

In our present study, all patients in the treatment group received a daily intake of probiotic-containing *Bacillus subtilis* HU58 (2 x10^{9} CFU/cap) by oral route for 7 days. A follow up on the 15th day showed statistically

Table 4: Studies conducted with *Bacillus subtilis*

Sr No.	Design of study	Probiotic and dose	Number of volunteers	Study reference
1.	Randomized, parallel, blind, with control, Multi-centric	*Bacillus subtilis* 3 x 10^9 CFU	95 patients with acute diarrhoea and 48 patients with chronic diarrhoea	Wang et al 2004
2.	Randomized but not double blinded	*Bacillus subtilis* 3 x 10^9 CFU	68 antibiotic associated diarrhoea patients	Li et al 2007
3.	Randomized but not double blinded	*Bacillus subtilis* 3 x 10^9 CFU	123 patients with acute diarrhoea	Chen and Zhu 2007
4.	Uncontrolled and not double-blind	*Bacillus subtilis* 3 x 10^9 CFU	47 patients with chronic diarrhoea	Lin 2007
5.	Randomized with active control but not double-blind	*Bacillus subtilis* 3 x 10^9 CFU	100 patients with chronic diarrhoea	Wang 2008
6.	Randomized with active control but not double-blinded	*Bacillus subtilis* 3 x 10^9 CFU	80 patients with diarrhoea and predominant IBS	Su *et al*. 2006

significant improvement. In a study by Wang and Jin in 34 patients with diarrhoea due to intestinal flora imbalance treated with *Bacillus subtilis* (Medilac-S) showed marked improvement in consistency of stool, abdominal pain, abdominal distention and fever[9]. Miller LE *et al*. demonstrated that *Bacillus subtilis* reduces chronic diarrhoea in 92% of people compared to the control group[11]. Other studies conducted on *Bacillus subtilis* probiotic on antibiotic-associated diarrhoea are listed in table 5[21].

Age factor did not play a major role in the frequency of AAD but patients suffering from illness had higher frequencies of diarrhoea. Patients treated with short duration of antibiotics had a low frequency of diarrhoea compared to patients treated for a long duration of antibiotics. There is evidence to suggest that the major risk factors associated with diarrhoea were long term use of antibiotics, multiple antibiotic doses, duration of hospital stay and infection control procedures[22]. Gut microbiota plays a very significant role in human health. The mechanism of action of gut microbiota is to prevent the colonization of pathogens, improve the immune system, production of gastrointestinal hormones and neuroactive substances. The fermentation of non-digestible carbohydrates at the level of the colon is done by gut microbiota which in turn produces short-chain fatty acids that provide health benefits[23]. The disruption of normal gut flora is caused due to the administration of antibiotics and results in diarrhoea. Antibiotics alter the carbohydrate metabolism and increase the antimicrobial activity in the colon[24]. Reduction in the metabolism of fermented carbohydrates in the colon leads to the reduction of short-chain fatty acids and an increase in the non-absorbable carbohydrate in the gut causing a rise in the osmotic pressure and reducing water absorption from gut leading to liquification of stools[25]. In addition to these effects, drugs which increase gut motility can exacerbate the situation and worsen or cause diarrhoea. Since antibiotics are one of the major causes of disruption of normal gut flora, probiotics are often recommended on the assumption that they would reduce the AAD[26]. Probiotics prevent diarrhoea by maintaining the gut flora by the fermentation of non-digestible carbohydrates and by inhibiting the growth of infection causing organisms[24]. Antibiotics could also affect the enteric nervous system during intestinal transit. They are also capable of reducing muscle tone and neuroeffector transmission to the intestinal muscularis mucosa[2].

In a recent study El Hage *et al*. reported that probiotics can be taken as a functional food supplement and could prevent AAD caused due to antibiotics as well as diarrhoea caused due to C. difficile[23]. Ruszczynski M et al in 2008 carried out a double-blinded, randomized, placebo-control study in 240 children having a common infection and receiving antibiotic therapy plus probiotic (n=120) or placebo (n=120) orally. Out of 120 children in the probiotic group diarrhoea occurred in nine (7.5%) patients, whereas 20 (17%) patients in the placebo group developed AAD[27].

Horosheva TV *et al* in 2014 conducted a randomized, double-blinded, placebo-control study on 308 patients randomly divided into 3 groups. Group 1 received probiotic containing strains B. *subtilis* 3 and B. licheniformis 31, Group 2 received only B. *subtilis* 3 and group 3 received placebo. In group 1 which received a mix of probiotic strains 9 out of 91 patients developed AAD. In group 2 which received a single strain of probiotic 7 out of 90 patients developed AAD and the highest incidence of AAD was observed in group 3 which received the placebo, 23 out of 90 patients[28].

Evans M *et al* in 2016 conducted a randomized, double-blinded, placebo-controlled trial in 146 healthy adults and evaluated the effectiveness of probiotics in managing antibiotic-associated diarrhoea. All study subjects were administered one capsule of amoxicillin plus clavulanic acid for 1-7 days. Participants in the study group further received probiotics for 1-14 days, while the control group received a placebo for 1-4 days. Follow up was done on day 22 and 63. An increase in bowel movement was seen in both groups but the duration of diarrhoea was less in the probiotic treated group[29]. Cuentas AM *et al* in 2017 carried out a study to evaluate the effect of *Bacillus subtilis* DE111 on 50 adults aged 18-65 years suffering from occasional constipation and/or diarrhoea. They were administered either with *Bacillus subtilis* DE111 ($1x10^9$ CFU) or placebo for 105 (it is 90) days. Questionnaires were conducted on days 1, 15, 45, 75, and 105. Improvement was observed in *Bacillus subtilis* DE111 consuming group compared to the placebo. No adverse effects were observed during the study[30].

In a 2017 study conducted by Zang *et al.* on 163 elderly patients who were exposed to antibiotics for a minimum of three days. In the study group 81 patients were administered with probiotic for 21 days twice a day. The control group only received the antibiotic. The incidence of antibiotic-associated diarrhoea was lower compared to the placebo group.

Conclusion

Antibiotic-associated diarrhoea is a common health care problem. Probiotic *Bacillus subtilis* HU58 offers an effective control for AAD. Probiotic *Bacillus subtilis* HU58 at a dose of 2 X 10^9 CFU/Cap once a day for 7 days effectively improved stool consistency in AAD patients compared to placebo. All patients showed good tolerability for the ingestion of Probiotic *Bacillus subtilis* HU58 capsules. Data of group receiving probiotic *Bacillus subtilis* HU58 was found to be statistically significant (p value< 0.0001) for stool consistency as compared to the group receiving placebo.

Probiotic *Bacillus subtilis* HU58 was found to be well-tolerated and safe. However, the therapeutic efficacy needs to be evaluated further in a larger sample size, with a placebo/active-controlled double-blind randomized multi-centric trial.

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