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Dysbiosis Gut Restored to Normal Symbiosis using Homemade Buttermilk from Buffalo Milk

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Abstract

Human gut is hosted with huge number of bacteria, microbiota maintain the normal healthy life of humans. Healthy human gut recorded 10^{12} of microbes/gm. of solid food in gut. Large intestine is storing near about 100 to 10000 microbial species in it. Normal microflora of gut changes when the external flora reaches to gut through contaminated food and water. Abnormal flora of gut causes dysbiosis which leads to cause flatulence, gases, abdominal pain and even cancer. Natural Core microbiota also reduces when antibiotic used to treat. In the present study, I collected the stool sample of 15 patients who were used RO filter water and get dysbiosis due consumption of contaminated street food and tap water. Stool sample of patient was analysed before and after the buttermilk treatment. This study found that, the stool sample before the buttermilk treatment recorded large number of Gram positive cocci and short rod as well as small number of Gram negative rods and bacteroides. After buttermilk treatment the stool sample revealed large number of Gram negative short rod and bacteroides. The growth pattern of dysbiosis gut sample was also evaluated. Before buttermilk treatment growth recorded slow on Mac-Conkeys agar and huge growth on Nutrient agar whereas after buttermilk treatment growth on Mac-Conkeys agar is huge and low on nutrient agar. Form this study it is conclude that buffalo buttermilk restore the normal microflora and improve the health.

Keywords: Microbiota, Dysbiosis, Gut, Antibiotics.

1. Introduction

Gastrointestinal track (GI) start at the mouth and end at the anus, the average adult host 500-2000 different microbial species in their gut, human gut is one of the most densely packed microbial ecosystem on our earth planet [1, 3]. Human large intestine found to contain about 10^{12} cell/gram of stool sample. Large intestine recorded near about thousands of different microbial species [4, 5.7]. The gut microbiota is very unique in every individual and somewhat similar in an indigenous population. The diversity of gut microbiota is also depends on the food and environment [2, 8, 9]. Human with microbiota is also called "superorganism" [6]. However, a recently revised study has suggested that the ratio of human: bacterial cells are actually closer to 1:1 [5]. Non vegetarian human recorded highest number of bacteroides in the gut whereas fibres based food consumer shows highest number of firmicutes in their gut [7]. In gut undigested food convert into some carcinogenic compound by certain bacteria. Some microbiota converts food material into acetic acid, butyric acid and acetate in the gut. This acid prevents the growth of some unwanted microflora in the gut [5]. In India since last few years, the use of UV and reversosmosis treated water used by middle and upper class societies which maintain specific core microbiota. India is developed country and potable water and food supply to every individual is still changelings, when the middle and upper-class society people travels to certain holy places their core biota challenges by the food and water at the holy place [9]. Commuters generally complain abdominal discomfort with the flatulence pain and diarrhoea. In this study 15 commuters stool sample was analysed and treated with buttermilk of buffalo. Buffalo buttermilk normalise the good biota and curved the patient within 3 days consumption of buttermilk.

2. Material and Method

i). **Participant Selection:** After obtaining informed consent and a total of 15 participants were selecting for the study. Faecal sample obtained before and treatment and after buttermilk treatment. About 1 gam of faecal matter collected in 50 ml screw cap bottle and brought to the laboratory and directs microscopic observation was performed using Gram 2staining.

ii). **Gram Staining:** Smear of faecal matter was prepared and primary crystal violet satin flood for 2 to 3 min, then mordent (Grams iodine) used for 30 second. CV- Iodine complex was decolourised with 95% ethanol and at the

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last safranin was used as counter stain for 1 minute and observation were carried out using oil immersion object.

iii). **Culture Media:** Mac-Conkeys agar and Nutrient agar was used for culturing and growth was characterised.

iv). **Preparation of Buttermilk:** Unique Starter culture was collected from housewife Arti chavan from Dhawari tanda Maharashtra state (India). About 250 ml of starter culture inoculated in 5 litter of sterilised milk and allowed to ferment at room temperature for 24 hr. after fermentation one bowl of culture used to feed the patient twice a day for three consecutive days.

3. Observation and Results

The stool samples collected before the treatment of buttermilk. The microscopic study of stool sample of all 15 patients showed that, large number of the Gram positive cocci and rod present in their large intestine (fig 1 table 1). Stool sample after the treatment of buffalo buttermilk revealed that stool sample replaces the gram positive cocci with large number of gram negative shorts rods (Fig 2 and table 2). Stool sample before treatment when cultivated on Mac Conkeys media and incubated at 37^0C for 24-48 hours showed very less growth whereas on Nutrient media at the same growth condition huge growth was recorded. (Fig 3 and 4). After the buttermilk treatment when stool sample cultivated at same cultural condition showed huge growth on Mac Conkeys media and less on nutrient media. (Fig. 5 and 6 and table 2.)

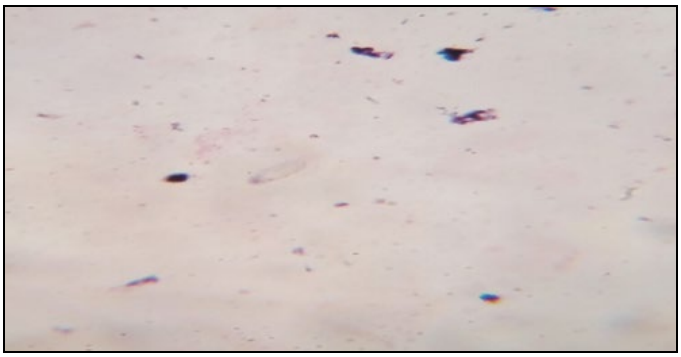

Fig 1: Gram staining

Table 1

Serial No. of Patient	Gram Staining of Stool Sample Before Buttermilk Treatment
1	Mostly gram positive cocci
2	Gram positive cocci and gram positive rod
3	Gram positive cocci
4	Gram positive cocci and rod
5	Gram positive cocci rod, gram negative cocci
6	Gram positive cocci
7	Gram-positive cocci, gram negative cocci
8	Gram positive cocci
9	Gram positive rod, cocci
10	Gram negative cocci and rod
11	Gram positive cocci and rod
12	Gram negative rods and positive cocci
13	Gram positive cocci
14	Gram positive cocci
15	Gram positive cocci

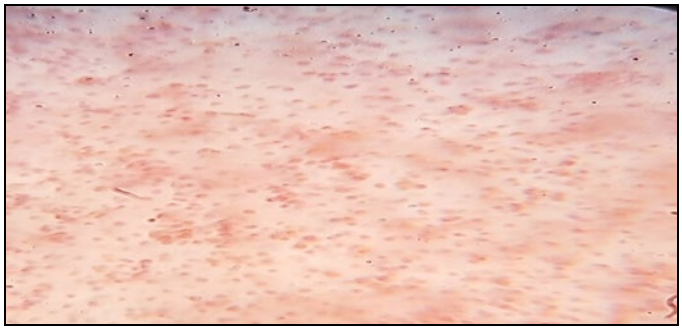

Fig 2: Gram staining

Table 2

Serial No. of Patient	Gram Staining of Stool Sample after Buttermilk Treatment
1	Gram negative short rod
2	Gram negative short rod and negative cocci
3	Gram negative cocci and rods
4	Gram negative rod and cocci
5	Gram negative rods and cocci
6	Gram negative cocci and rods
7	Gram negative short rods
8	Gram negative rods
9	Gram negative short rod
10	Gram negative cocci and rod
11	Gram positive rods
12	Gram negative short rods
13	Gram negative rod and cocci
14	Gram negative rod and cocci
15	Gram negative rods and cocci

Fig 3: Growth on Mac-Conkeys agar

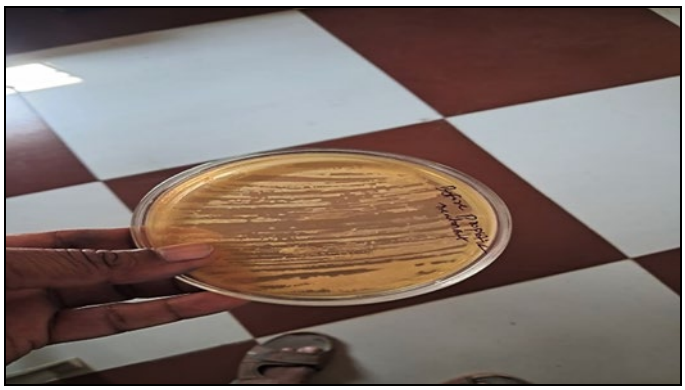

Fig 4: Growth on Nutrient agar

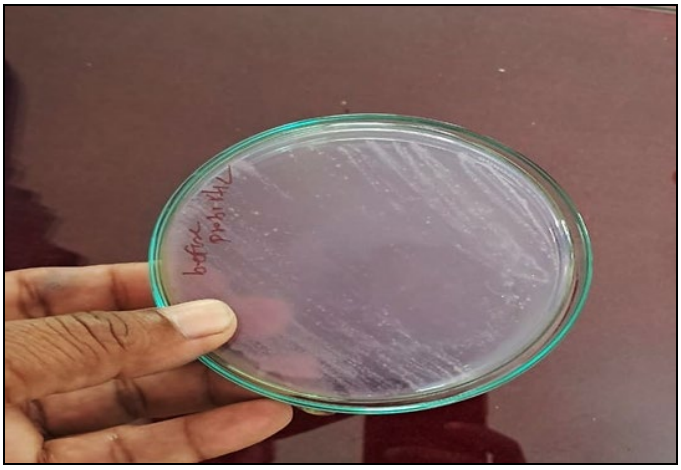

Fig 5: Growth on ma-Conkeys Agar

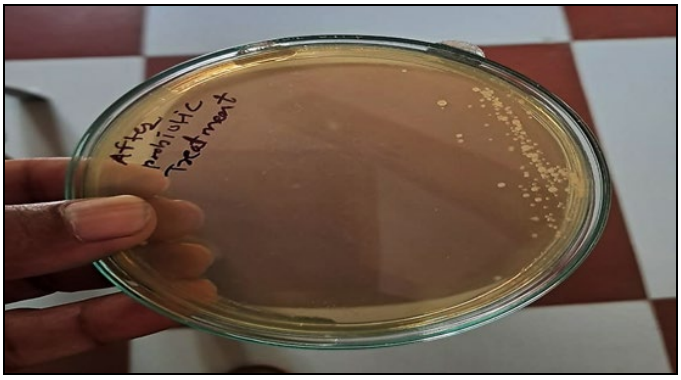

Fig 6: Growth on Nutrient agar

4. Discussion

Microflora are very useful for the human survival, after three year of the age these microbiota established on the boy of the host [1] it is estimated that human has harbour 10^{14} microbial cells all over the body of human. Microbiota harbouring organisms are called superorganism. Genome of microbiota and humans are combinedly called microbiome. [4] microbiota develops the immunity of host as well as prevents the formation of cancer. Microbiota in gut produces the acetic and butyric acids which maintain the gut health. Some studies reported that gut of obeys people have large number of gram positive and can led to cause cancer and obesity [5]. Gram negative bacteria in gut maintain the health status of the human and synthesises vitamin B12, K and butyric acids and are helpful [7]. If, this microbiome disturbed by exotic microbiota gastric abnormality occurs. Based on data our study find out that 15 patient who travels holy places and acquired exotic gram positive cocci in their gut, which caused the pain and gastrointestinal disturbance, flatulence and loss of appetite. Experimental study found that when buffalo buttermilk feeds to them it normalise the normal microflora belonging to gram negative bacteria. Buttermilk enriched with probiotics of native species that produces certain compounds which inhibit the growth of gram positive cocci. The study of Jiang Zhu 1, Yuping Zhu and GangSong reports Effect of Probiotic Yogurt Supplementation (Bifidobacterium animalis ssp. lactis BB-12) on Gut Microbiota of Female Taekwondo Athletes and Its Relationship with Exercise-Related Psychological Fatigue and report that probiotics are useful to combat the health issues [5]. Our finding is similar in accordance with their finding. A review article on the role of normal microbial flora in human body by Suneeta Singh, Anjali Rajput, Satyendra Singh & Anshu Kumar Singh also reported the importance of microflora for developing immunity [6]. Prescott's microbiology by Joanne *et al* reported that normal microflora is depends on the surrounding environment [8]. An antibiotic disturbs the microflora remarkably and change the health status. In the light of the above data we cure the abdominal discomfort in 15 patients using the buffalo buttermilk. Buttermilk enriched with lactic acid bacteria and certain unknown compound in it. Further study needs to revels the fermentation product in the butter milk, so that it can be a better remedy to restore the normal microflora.

5. Conclusion

The homemade butter milk of buffalo milk contains probiotics and certain ferment products into it. These probiotics combat with exotic species which causes the flatulence, pain and indigestion and normalise the normal flora of gut and restores the natural symbiosis in human gut

6. **Conflicts of Interest:** The authors declare no conflict of interest

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