**Evaluating the effect of prebiotics in the rehabilitation of gut microbiota after antibiotic therapy in rats**

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**Abstract**

The present study evaluated the effect of galacto-oligosaccharides (GOS)on gut microbiota after antibiotic treatment given two times in a day. Four groups were made having six rats in each group. G1 was a control group fed on a basal diet. While, the remaining were treated in groups given antibiotic and GOS separately and also in combination as in G2. The dose of antibiotic and GOS was calculated by HED (Human Equivalent Dose) formula. Fecal samples were analyzed at the interval of five days for bacterial population especially *Bifidobacterium* spp., *Lactobacillius* spp., *E. coli* and total plate count using selective media. The results indicated that the growth of *Bifidobacterium* spp. and *Lactobacillius* spp. depended on GOS and antibiotic dose. The combination of GOS-Cephalexin is mostly of interest because due to the antibiotic the results of *Bifidobacterum* spp. and *Lactobacillius* spp. were decreased while on GOS consumption the growth of such species is increased. The results of G3 showed that the number of colonies of *Bifidobacterium* spp. and *Lactobacillius* spp. was significantly higher than G2 on 5th day. Furthermore, the recovery rate was faster as compared to other groups. This research suggested that intake of GOS during antibiotic treatment significantly strengthen the microbiota by increasing the population of *Bifidobacterium* spp. and *Lactobacillius* spp. as well as reduce the number of *E. coli* that shows resistance to many antibiotics.

**Keywords:** Galacto-oligosaccharide, *Lactobacillus*, *Bifidobacterium*, *Escherichia* *coli,* Food Microbiology, Cephalexin.

**Introduction**

Human beings and other multicellular organisms have a close alliance with micro-organisms. It is stated that the human gastrointestinal tract consisted of trillions of cells, an estimated number is 1014 bacterial cells ([Sekirov *et al*. 2010](#_ENREF_21)). Immediately, after birth bacteria continue to grow until the first year of life span. Afterward, they remain ([DuPont 2016](#_ENREF_3)). It is considered that when the gut microbiota is going to be established, higher than 50 diverse phyla and more than 500 bacterial species present in human gut microbiota. However, the exacted amount of that species and variability among individuals is still needed to be categorized ([Rastall 2004](#_ENREF_17)). These features are highly hooked on a diet, lifestyle as well as host genotype ([Hopkins *et al*. 2001](#_ENREF_7); [Zoetendal *et al*. 2001](#_ENREF_29)).

It is true that from the past 80 years antibiotics saved millions of life by destroying pathogens but the drawback of these also killed beneficial bacteria. In 2015, 50,000 deaths occurred just because of antibiotic-resistant pathogens. In 2025, this value has been increased up to 10 million deaths per year worldwide. So, all the above data show that we are reaching the end of an antibiotic era **(**[Grande-Bretagne 2014](#_ENREF_6))**.** However, due to excessive use of antibiotics, we have lesser control on pathogens. Eradicating all pathogens due to their variability is impossible. Hence, attention has turned to the gut microbial ecosystem that acts as an “effective barrier” against pathogens ([Stefka *et al*. 2014](#_ENREF_24)).

In third world countries, there is an additional use of antibiotics that disrupt the human gut microbiota and leads to various problems. Such as highest use of amoxicillin and clindamycin lead to antibiotic-associated diarrhea and it is only happening due to an imbalance of microbiome ([McFarland 2008](#_ENREF_13)). Increased evidence shows that the said drugs persuaded changes in the configuration of normal gut system and cause of various diseases. Many evidences support this statement. For example, broad-spectrum medicines, particularly beta lactam, target the vitamin K producing bacteria ([Shevchuk 1992](#_ENREF_22)). Furthermore, they also linked with the inappropriate immune system ([Teo *et al*. 2015](#_ENREF_25)). The solution of all these problems is to manipulate the gut microbiota and it can be managed by the various ways such as modify the resource supply through controlled diet, by the addition of supplements and the use of prebiotics.

Prebiotics can be defined as: “selectively fermented ingredient that results in specific changes, the configuration or activity of the gastrointestinal microbiota providing beneﬁts upon

host health”. It is an emerging belief that these are beneficial regarding balanced the gut micro-organisms. Unlike probiotics, these are easy to ingest. These can be categorized as dietary fiber or indigestible carbohydrates ([Macfarlane *et al*. 2006](#_ENREF_12)). This acidic environment is favorable for the growth of beneficial bacteria such as *Bifidobacterium* spp. and *Lactobacillius* spp. Contrary to it, creates a negative impact on the growth of pathogenic bacteria ([Sako *et al*. 1999](#_ENREF_18)). Hence, the gut microbiota performs a wide variety of metabolic activities that are essential for the host’s metabolism.

Prebiotics are vital because they have many beneficial effects on human health. They provide substantial physiological effects depending on the configuration and activities of intestinal microbiota, both in the lumen and at mucosal layer ([Keeney and Finlay 2011](#_ENREF_9)). Many types of carbohydrates retain prebiotic properties but have been best accepted for indigestible oligosaccharides, i.e., galacto-oligosaccharides ([Gibson *et al*. 2005](#_ENREF_4); [Tuohy *et al*. 2005](#_ENREF_26)). The functional food components of prebiotics are GOS (galacto-oligosaccharides), FOS (fructo-oligosaccharides) and gluco and xylo-oligosaccharide ([Wasilewski *et al*. 2015](#_ENREF_28)).

The use of GOS in research is to manage the gut system by rehabilitation of beneficial bacteria especially *Lactobacillus* spp. and *Bifidobacterium* spp. that are destroyed by the antibiotic treatment.

**Materials and Methods:**

All the chemicals and materials were analytical grade and purchased from local market of Lahore, Pakistan otherwise stated below. Moreover, the fecal sample collected through rats and move in laboratory carefully through sample kit. Finally, the microbial analysis of the fecal samples and the colony counting was done in the laboratory of food science and human nutrition.

**Subjects**

Twenty-four adult albino rats were selected and housed in stainless steel cages in the animal shed at University of Veterinary and Animal Sciences, Lahore. The rats were kept in the environmentally controlled room with a temperature of 24 ± 5 ℃, under a 12 h light: 12 h dark cycles and given free access to water and food. Rats were allowed to acclimatize in the new environment for one week. The experimental procedure used in this research was approved by the University Ethics Committee for Animal Research.

**Prebiotic Source:-**

Galacto-oligosaccharide (GOS) was used as a source of prebiotic. The company of Friesland Campina Domo imported Vivinal® GOS powder. It comprises of 97 % dry matter (milk solids) 3 % moisture; 69% galacto–oligosaccharides , 23% lactose, 5% monosaccharides (glucose and galactose) which is the purely high product of galacto- oligosaccharide. It was kept in air tight bag and stored under the lab of Department of Food Science and Human Nutrition, University of Veterinary and Animal Sciences (UVAS) Lahore.

**Experimental Design:**

Twenty four healthy rats were randomly divided into four groups having six rats in each group. Group G1 was a control normal that was fed on a basal diet while the remaining groups were treated groups. Groups G2 to Group G4 were given cephalexin antibiotic for five days. Moreover, Group G3 was treated with the combination of GOS and antibiotic for five days, but GOS was continued for further fifteen days. In Group G4, firstly antibiotic was given for specific duration and after that GOS was given for the rest period. The amount of GOS and antibiotic was calculated by the Human Equivalent Dose. HED = animal dose in mg/kg x (animal weight in kg/ human weight in kg)0.33. The amount of GOS and antibiotic was 158 mg and 9 mg respectively. The fecal samples were collected from rats at 5th day time interval and analyzed for bacterial population especially *Bifidobacterium* spp., *Lactobacillius* spp*.*, *E. coli* and total plate count using selective media.([Ladirat *et al*. 2014](#_ENREF_10)). A half gram of fecal sample was taken into test tubes which were filled with phosphate buffer solution having a (pH 6.8).

**Procedure for Bacterial Enumeration**

**Microorganisms and Media**

*Lactobacillius* spp., *Bifidobacterium* spp. and *Escherichia* *coli* were counted before and after the treatment as indicator organisms. These bacteria were grown on their selective media. *Lactobacillus* selection agar base, Bifidobacterium agar, modified (De Man, Rogosa and Sharp agar) and (Eosin Methylene Blue gar) respectively. Incubation was done of *Bifidobacterium* spp. in an anaerobic jar while *Lactobacillus* spp., *E. coli* and total plate count in aerobic condition at 37 °C. For an accurate count, the number of colonies per plate should not exceed 300 nor be less than 30 colonies ([Schillinger and Lücke 1989](#_ENREF_20)).

**Statistics**

Statistical analysis was conducted with the Statistical Package for Social Science (SPSS for Windows version 18, SPSS Inc., Chicago, IL, USA). Data were presented as mean ± S.D. The data were analyzed using One-way Analysis of variance (ANOVA). The group differences were compared by the Duncan’s Multiple Range Test. The difference was considered significant at *P ≤* 0.05. The interaction was calculated by Two-way Analysis of variance (ANOVA).

**Results**

***Bifidobacterium* Enumeration**

The colonies of *Bifidobacterium* spp of four different groups were counted at intervals of 5, 10, 15 and after 20 days. The log value of CFU/ ml of control and treated groups of *Bifidobacterium* spp are given in table 1.

**Table 1** Mean log value of *Bifidobacterium* spp. at different time intervals

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Groups | 0 day | 5 day | 10 day | 15 day | 20 day |
| G1 | 10.23±0.033a,A | 10.21±0.022a,B | 10.25±0.075a,A | 10.31±0.077a,A | 10.29±0.064b,A |
| G2 | 10.32±0.049a,A | 6.30±0.083c,D | 6.57±0.055d,C | 7.39±0.058c,B | 7.39±0.058d,B |
| G3 | 10.31±0.043a,B | 8.22±0.056b,D | 9.38±0.079b,C | 10.37±0.060a,B | 11.28±0.042a,A |
| G4 | 10.27±0.112a,A | 6.28±0.061d,E | 7.37±0.046c,D | 8.51±0.032b,C | 9.62±0.048c,B |

**Legend:** All the values of *Bifidobacterium* spp. at different time intervals are mentioned in log numbers and are means ± standard deviation for six albino rats. The means sharing the same superscript small alphabets in a column are statistically similar to each other, showing the effects of days of four groups. Moreover, means sharing the same superscript Capital alphabets in a row are statistically alike to each other, showing the effect of treatment at the specified time.

The number of colonies of *Bifidobacterium* spp. at 5th day shows that there was a reduction of colonies when antibiotic was given to treated groups. However, less reduction in colonies was observed in G3 because of GOS. The number of *Bifidobacterium* spp. was highly recoverable in G3 shows that GOS consumption from 5th day to 20th day strengthen the gut microbiota. The interaction is less than 0.05 indicated that interaction between time and groups are significant.

***Lactobacillus*  Enumeration**

The colonies of *Lactobacillus* spp. of four groups were counted at intervals of 5, 10, 15 and after 20 days were given in table 2. The interaction is less than 0.05 indicated that interaction between time and groups are significant.

**Table 2** Mean log value of *Lactobacillius* spp. at different time intervals

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Groups | 0 day | 5 day | 10 day | 15 day | 20 day |
| G1 | 10.31±0.071a,A | 10.30±0.135a,A | 10.30±0.135a,A | 10.26±0.107b,A | 10.31±0.016b,A |
| G2 | 10.29±0.058a,A | 6.28±0.119c,D | 6.47±0.098d,C | 7. 40±0.083d,B | 7.40±0.083d,B |
| G3 | 10.29±0.062a,C | 8.56±0.057b,E | 9.26±0.096b,D | 10.38±0.050a,B | 11.27±0.038a,A |
| G4 | 10.27±0.103a,A | 6.21±0.089c,D | 7.32±0.048c,C | 8.50±0.0250c,B | 8.50±0.025c,B |

**Legend:** All the values of *Lactobacillius* spp. at different time intervals are mentioned in log numbers and are means ± standard deviation for six albino rats. The means sharing the same superscript small alphabets in a column are statistically similar to each other, showing the effects of days of four groups. Moreover, the means sharing the same superscript Capital alphabets in a row are statistically alike to each other, showing the effect of treatment at the specified time.

On 5th day, the numbers of colonies were significantly less from zero-day. As well as lower number of *Lactobacillius* spp. were observed in G2 and G4 that just treated with the antibiotic. Similarly, on 10th day all the groups were significantly different from each other. Furthermore, when samples were analyzed on 15th day the G3 has been recovered and at 20th day it has been increased from G1. Although, the colonies were high in G4 that given GOS after antibiotic as compared to G2 but still not recovered at 20th day. The interaction is less than 0.05 indicated that interaction between time and groups are significant.

***E. coli* Enumeration**

The log number of colonies of various groups of *E. coli* is mentioned in table 3. Six different replicates were used in the experiment.

**Table 3** Mean log value of *E. coli* at different time intervals

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Groups | 0 days | 5 day | 10 days | 15 days | 20 days |
| G1 | 11.37±0.064a,A | 11.33±0.098a,A | 11.33±0.098a,A | 11.28±0.124a,A | 11.35±0.063a,A |
| G2 | 11.37±0.051a,A | 7.27±0.063b,D | 7.58±0.043b,C | 8.26±0.110b,B | 8.26±0.110d,B |
| G3 | 11.37±0.061a,A | 7.27±0.063b,D | 7.27±0.063d,D | 8.21±0.072b,C | 9.28±0.059c,B |
| G4 | 11.28±0.052b,A | 7.25±0.079b,E | 7.43±0.057c,D | 8.29±0.053b,C | 9.47±0.080b,B |

**Legend:** All the values of *E. coli* at different time intervals are mentioned in log numbers and are means ± standard deviation for six albino rats. The means sharing the same superscript small alphabets in a column are statistically similar to each other, showing the effects of days of four groups. Moreover, the means sharing the same superscript Capital alphabets in a row are statistically alike to each other, showing the effect of treatment at the specified time.

This table illustrates that when antibiotic was given to G2 to G3 the number of colonies was significantly decreased from normal. On 10th day the G3 has the lowest number because of GOS utilization that started from the 5th day. However, no group was able to many such bacteria’s colonies till the 20th day that meet with the control group. The interaction is less than 0.05 indicated that interaction between time and groups are significant.

**TPC Enumeration**

The mean log values of TPC in cfu/ml are mentioned in table 4.

**Table 4** Mean log value of TPC at different time intervals

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Groups | 0 days | 5 day | 10 days | 15 days | 20 days |
| G1 | 12.30±0.154a,A | 12.27±0.105a,A | 12.27±0.105a,A | 12.34±0.032a,A | 12.36±0.067a,A |
| G2 | 12.36±0.059a,A | 8.18±0.149c,D | 8.56±0.057d,C | 9.20±0.129d,B | 9.20±0.129c,B |
| G3 | 12.32±0.046a,A | 9.26±0.096b,D | 10.29±0.062b,C | 11.28±0.042b,B | 12.31±0.033a,A |
| G4 | 12.30±0.42a,A | 8.24±0.071c,E | 9.41±0.069c,D | 10.31±0.064c,C | 11.36±0.045b,B |

**Legend:** All the values of total plate count (TPC) at different time intervals are mentioned in log numbers and are means ± standard deviation for six albino rats. The means sharing the same superscript small alphabets in a column are statistically similar to each other, showing the effects of days of four groups. Moreover, the means sharing the same superscript Capital alphabets in a row are statistically alike to each other, showing the effect of treatment at the specified time.

Results were counted for four different groups on the 5th day it showed that the healthy group (control) has the highest number of species significantly as compared to other groups. However, G3 has a significantly lower number of colonies from control while greater as compared to G2 and G4. The recovery rate was first observed in G3 on the 20th day while the other treated groups did not rehabilitate their gut microbiota at the same day.

**Discussion**

Antibiotics influence the human health by creating imbalances of microbes present in the human gut. The use of antibiotics in a high number from childhood to adult cause short and long-term consequences for our health ([Langdon *et al*. 2016](#_ENREF_11)). The human gut system can be deviated from normal system due to antibiotic usage ([Van der Waaij *et al*. 1971](#_ENREF_27)).

The purpose of this study was to enquire whether the response of prebiotic dosage after the antibiotic dose is valuable for the recovery of beneficial bacteria such as bifidobacteria and lactobacilli. During the research, the effect of treatments on various types of bacteria like *Bifidobacterium* spp.*, Lactobacillius* spp.*,* Total Plate Count and *E. coli* species were analyzed.

When the effects of the broad-spectrum antibiotic on the gut microbiome were analyzed, it is stated that it definitely affects the gut microbes. Because In Group 2 and Group 4 that were treated with antibiotic treatment for five days the number of *Bifidobacterium* spp. and *Lactobacillius* spp. were significantly reduced from the normal (*p*< 0.05). It clearly shows that the treatment of antibiotic for the 5th day majorly disturbed the configuration and reduce the diversity of gut microbiota that was concordant with previous studies ([Panda *et al*. 2014](#_ENREF_15)).

In earlier research conducted on 84 newborns (49 newborns were kept in a normal group while remaining were categorized as Intrapartum Antibiotic Prophylaxis (IAP) group) to check the effect of IAP on gut microbiota of infants. This research stated that the newly born babies treated with the antibiotic have a significantly lower number of *Bifidobacterium* spp. (log value = 6.01) as compared to the normal group (log value = 7.80). Decreased vertical transmissions of *Lactobacillus* spp. were also observed in IAP treated mothers ([Corvaglia *et al*. 2016](#_ENREF_2)).

Moreover, another study stated that antibiotic treatment for more than 5 days in infants leads to severe problems such as necrotizing, sepsis, etc. Prolonged treatment also changes the composition of gut microflora by enhancing the Proteobacteria and reducing the colonies of beneficial bacteria such as bifidobacteria ([Langdon *et al*. 2016](#_ENREF_11)).

Antibiotics kill some beneficial species, for example, *Bifidobacterium* spp. and *Lactobacillius* spp. After antibiotic treatment the number of colonies reduced. The present study confirms previous data according to which the fecal count of *bifidobacterium* spp and *Lactobacillius* sp. is reduced by antibiotic is given for 3 to 7 days ([Newton *et al*. 2013](#_ENREF_14)).

Additionally, the increased resistance and the severe effects of broad-spectrum antibiotics on gut microbiome stated that it must be complemented with such interventions that would help in stimulating the beneficial bacteria after destruction.

One such advancement is modifying the diet such as the use of prebiotics, e.g. fructo-oligosaccharides (FOS) and galactooligosaccharides (GOS). They confer many health benefits to the host as well as protect from external and internal pathogens but not *Clostridium difficile* ([Gibson *et al*. 1995](#_ENREF_5); [Hopkins and Macfarlane 2003](#_ENREF_8)).

In present study when G3 were given both antibiotic and prebiotic, then the number of colonies of *Bifidobacterium* spp. and *Lactobacillius* spp. were significantly higher than G2 at 5th day. Furthermore, the recovery rate was faster as compared to other groups.

In Langlands *et al.* study volunteers were given 7.5 g of oligofructose and the same amount of inulin. When results were matched with the normal group it was observed that the number of *Bifidobacterium* spp. and *Lactobacillius* spp. were notably increased from normal ([Langdon *et al*. 2016](#_ENREF_11)).

Similarly in another study on a human when treated with 10g of trans-galactooligosaccharides was also mentioned that there was increased number of *Bifidobacterium* spp. by consuming such type of prebiotic and also modify the gut flora by increasing fermentation metabolism in the colon. Moreover, inulin type fructans when given to mice it also correlated with the above-mentioned studies showing a higher number of bifidobacteria count ([Patel and DuPont 2015](#_ENREF_16)).

When results of *Lactobacillius* spp. of four groups were studied, it showed different results at different time intervals. On the 5th day the bacterial colonies of G2 and G4 showed disruption in gut microflora due to antibiotic treatment. However, it showed some difference with G3 as some species of this group high in values due to the consumption of GOS. The results were observed and also calculated the number of colonies at 10th, 15th and 20th day sampling. It was noticed that the results of G3 were significantly same with G1 at 15th day. While, G4 also showed some recovery as there number of colonies were greater than G2. It indicated that beneficial bacteria were highly recoverable due to the bifidogenic effect of GOS. Moreover, G2 was not recovered till at 20th, but it may be increased after two weeks because according to a study the destruction is permanent the *Bifidobacterium* spp. count was attained its original position after one month ([Corvaglia *et al*. 2016](#_ENREF_2)).

During antibiotic therapy, it was noted that the utilization of prebiotics (GOS) was helpful in the rehabilitation of micro-biota. After the study, it was observed that due to GOS consumption the recovery rate of *Bifidobacterium* spp. and *Lactobacillius* spp. was faster.

Researches above also stated that the development of beneﬁcial bacteria like *Biﬁdobacterium* spp. and *Lactobacillus* spp.can grow fast along with 3 to 4 types of antibiotics (Stephanie *et al*. 2013; Brunser *et al*. 2006). This is because the GOS produce short chain fatty acids that can reduce inflammation in the colon and enhance the amount of valuable bacteria such as *Biﬁdobacterium* spp and *Lactobacillius* spp ([Scheppach and Weiler 2004](#_ENREF_19)).

The results of *Bifidobacterium* spp. showed that the groups treated with GOS were recoverable. Recovery rate was faster in G3 that was given GOS with antibiotic as compared to G4. While no recovery was examined in G2 the recovery rate is so much slow at the 20th day it did not meet with microflora of the normal group.

On 5th day it was observed that demise of microbiome took place with the use of antibiotics when it was compared with G1. However, the reduction of *Bifidobacterium* spp. colonies was also occurred in G3, but bifidobacteria and lactobacilli count were comparatively higher than G2 and G4.

We had also checked the results of 10th and 15th day. It was seen that when results were counted at the 15th day the number of bifibacterial colonies of G3 that were given antibiotic and prebiotics side by side, were significantly same with group G1. It indicated the recovery of probiotic (bifidobacteria and lactobacilli) by using prebiotic (GOS).

When a number of colonies were counted at the 20th day, it illustrated that the number of colonies in group 3 was notably higher from normal. On the other hand, G2 had lowest colonies showed that did not recover back to its position till the 20th day.

Two other protocols were also observed as total plate count and *E. coli*. Cephalexin is active against some gram negative species such as *E. coli*, *Morexella*, etc ([Arslanoglu *et al*. 2008](#_ENREF_1)). The study indicated that during antibiotic treatment the number of colonies is reduced in group 2. The number of colonies enhanced on 10th, 15th and 20th day in the same group. However, the rising level of *E. coli* was significantly lower in group 3 and group 4 as compared to G2 because these favored with GOS. The previous studies relate with the present research by stated that GOS act as an anti-adhesive agent against *E. coli* and finally suppress their number in microflora ([Shoaf *et al*. 2006](#_ENREF_23); [Patel and DuPont 2015](#_ENREF_16)).

When TPC results were counted it also showed that colonies depend on antibiotic and GOS consumption. TPC amount in group 3 was significantly same with group 1 as P value is less than 0.05. Moreover, the total plate count in group 2 was reduced by the use of an antibiotic.

Furthermore, the change in microbiota were mainly observed for the beneficial species like *Bifidobacterium* spp. and *Lactobacillius* spp. that provide a crucial role in human health. Change in normal flora is usually associated with the usage of GOS and also depend on an antibiotic. While, total plate count was expected to increase on GOS usage and considered to be decreased by the treatment of antibiotic cephalexin.

There was no remarkable change observed in *E. coli* species because it shows resistivity 91.7% to cephalexin that belongs to the first generation of cephalosporin ([Arslanoglu *et al*. 2008](#_ENREF_1)).

**Conclusion**

Antibiotics are widely used to kill pathogenic bacteria that have adverse effects on human health. It has been observed that antibiotics cause an imbalance of microbes because these are not only targeting the pathogens but also suppress the growth of beneficial bacteria. Beneficial bacteria like *Bifidobacterium* spp. and *Lactobacillius* spp. conquer many benefits for human health. That is why there is a need to rehabilitate these valuable microorganisms, especially after antibiotic treatment. The important approach in this regard is the use of galacto-oligosaccharides (GOS). GOS considered as a functional component of prebiotics. Prebiotics are considered to have a beneficial effect on the disordered microbiota. The overuse of broad-spectrum antibiotic leads to the resistance that is a worldwide issue. So the modification of microbiota is essential that protect against pathogens as well as disease. This study suggested that intake of 8g of GOS by a human during antibiotic treatment significantly strengthen the microbiota by increasing the population of *Bifidobacterium* spp. and *Lactobacillius* spp. as well as put down the number of *E. coli* that shows resistance towards many antibiotics. This study suggested that 8g of GOS strengthen the microbiota imbalanced by 500 mg of antibiotic given two times in a day.

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