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CHARACTERIZATION OF BIFIDOBACTERIUM SPECIES ISOLATED FROM GUT MICROBIOTA OF VARIOUS AGE GROUPS

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ABSTRACT

Background: Bifidobacteria is a group of anaerobic non sporing gram positive bacilli with bifid appearance. They are present as normal flora in the intestinal tract, mouth and vagina and constitute the major component of normal human microbiota of the gut with probiotic potential. Despite the probiotic activity, sporadic cases of adverse effects due to Bifidobacteria were also reported. The composition of Bifidobacteria species has been reported to differ between stages of life. **Methods** :233 faecal specimen were collected from students, staff and their family members of different age groups without any gastrointestinal symptoms, processed anaerobically for the isolation of Bifidobacteria and subjected to Biochemical characterization. The doubtful isolates were subjected to Maldi Tof Analysis. **Results:** Of the 233 faecal specimen screened for Bifidobacteria, 123 strains were obtained, predominated by B.longum from all age groups. B.breve and B.adolescentis were found only in the age group 0-10. B.catenulatum was also detected in the paediatric group, mostly from 6-10 years and also from a 13 year old child. 10 cases of B.dentium was isolated only from age group between 31-50 and one from above 80 years. **Conclusions** : The present study investigates the gut makeup of Bifidobacterium species in different age groups by culture, biochemical characterization and Maldi Tof. Such reports are lacking from our country. The results of the study are comparable with the reported articles in the literature.

KEYWORDS : Bifidobacteria, probiotic, anaerobic, gut make up , Microbiota

INTRODUCTION

Bifidobacteria is a group of anaerobic non sporing gram positive bacilli and the name is derived from the typical bifid appearance. They are present as normal flora in the intestinal tract, mouth and vagina. Microbial consortium of the Gastrointestinal tract of breastfed infants is often dominated by Bifidobacteria[1] The initial colonization of gut depends on several factors such as mode of delivery, initial diet, hygienic practices and antimicrobial exposure. The species of Bifidobacteria decreases in adults although it remains relatively stable. B.breve was found to be predominant in newborns and exhibit an inverse relationship with B.longum in the adult population.[2]

As they are present as major component of normal human microbiota of the gut, it has been reported to possess probiotic potential according to some investigators. Bifidobacteria strains were found to exhibit a protective effect against infantile diarrhea caused by Rota virus and other enteric infections.[3] By employing, *in-vivo* animal experiments in mice using Probiotic strains, Babenko et.al demonstrated the anti Staphylococcal activity, elimination of S.aureus and establishment of a normal & stable vaginal microflora.[4] Another species B.longum had been reported to have a positive effect for reduction of Lactose intolerance in certain individuals.[5] Mitsuoka et.al compared the faecal flora of elderly persons in rural and urban areas of Japan and observed that the number of Bifidobacteria & C.perfringens were found to be inversely proportional in elderly population.[6] B.longum and B.breve were shown to prevent DNA induced damage by carcinogens.[7] In spite of the probiotic potential, Sporadic cases of adverse effects due to Bifidobacteria were reported by certain investigators, including invasive infections, especially in the immunocompromised hosts.[8-10]

The composition of Bifidobacteria species has been reported to differ between stages of life. Recent studies using molecular techniques have also indicated that there are differences in the composition of the gut microbiota between infants, adults and elderly population.[11] As no reports on characterization and species distribution in various age groups are available from our country in this aspect, the present study investigates the gut makeup of Bifidobacterium species in different age groups by culture, biochemical characterization and Maldi Tof.

MATERIAL & METHODS:

The present cross sectional study was conducted in a tertiary care hospital in Mangalore, Karnataka for a period of 1 year (January - December, 2019 sanctioned by Institutional Ethics Committee (FMMCIEC/CCM/12/2019)

Sample collection and transport: The specimen were collected from students, staff and their family members of different age group with no trace of intestinal pathogens or any gastrointestinal symptoms. Based on the sample size calculation, 233 specimen were subjected to the analysis of Bifidobacteria species . The faecal samples were collected in sterile wide mouthed containers and transported to the Research Laboratory for further processing without delay.

Microbiological Analysis: The faeces samples were inoculated directly onto Brucella Blood Agar, Bifidobacterium Selective Agar and HiChrome Bifidobacteria agar for the isolation of Bifidobacteria. The sample was also inoculated in a Robertsons Cooked Meat medium (RCM) as a supportive medium. Following an incubation period of 24 hours at 37^{0}C, faecal specimen in RCM was inoculated onto the above mentioned media and proceeded like direct specimen. The plates were placed in BD Gas Pak Jar with GasPak EZ sachet (anaerobe gas generating pouch system with indicator) or in Don Whitley Anaerobic workstation.

After an incubation of 72 hours, the characteristic small white colonies were presumptively identified as Bifidobacterium and were confirmed with gram stain (Y shaped gram positive bacilli with bifurcated ends). The colonies of Bifidobacteria grew as bluish green (B.infantis) and reddish pink with a halo zone (B.breve) on HiChrome Bifidobacteria agar. The biochemical characterization included catalase, Indole and Carbohydrate fermentation tests, using Viande-Levure broth as the basal medium.[12] When the biochemical reactions were doubtful, the colonies were subjected to Maldi Tof Analysis (Bruker, Daltonics, Germany) by Extended Direct Transfer technique employing 70% formic acid and 1.0µL of matrix solution, α-Cyano-4-hydroxycinnamic acid (HCCA).

RESULTS:

Of the 233 faecal specimen screened for Bifidobacteria, 123

strains were obtained, predominated by B.longum from all age groups (45 ie. 36.6) followed by B.bifidum (38 30.9%)) . Details are given in **Table -1**

Table -1

Sl.No	Species of Bifidobacteria	No	%
1	B.longum	45	36.6
2.	B.bifidum	38	30.9
3	B.adolescentis	08	6.5
4.	B.breve	12	9.8
5.	B.catenulatum	09	7.3
6.	B.dentium	11	8.9
	Total	123	100.0

Biochemical characterization of Bifidobacteria species was done according to Wadsworth Manual. **Table- 2.** As few of the biochemical reactions were variable, confirmation was done by Maldi Tof analysis.

Table 2

Species	Glucose	Lactose	Maltose	Arabinose	Cellobiose	Glycogen	Mannose	Sucrose	Salicin	Sorbitol	Starch
B.breve	+	+	+	-	+-	V	+	+	+	V	V
B.longum	+	+	+	+	-+	V	V	+	-	-	-
B.bifidum	+	+	+	-	-	-	-	-W	-	-	-
B.catenulatum	+	+	+	+-	+	-	-	+	+	+	-
B.dentium	+	+	+	+	+	+	+	+	+	-	+
B.adolescentis	+	+	+	+	+	+	V	+	+	V	+

All Strains were Catalase and Indole negative & fermented Glucose, Lactose and Maltose. **V-** variable reaction +- most strains positive -+most strains negative **-W** most strains negative, some weakly positive.

Distribution of Bifidobacteria according to various age groups is shown in **Table 3** . As shown in the Table, B.longum was isolated in higher numbers from all age groups. B.breve and B.adolescentis was found only in the age group 0-10. B.catenulatum was also detected in the paediatric group, mostly from 6-10 years and also from a 13 year old child.10 cases of B.dentium was isolated only from age group between 31-50and one from above 80

Table 3

Age	B.longum	B.bifidum	B.adolescentis	B.breve	B.catenulatum	B.dentium
0-5	3	-	4	10	2	-
6-10	3	-	4	2	6	-
11-20	8	4	-	-	1	-
21-30	12	11	-	-	-	-
31-40	2	8	-	-	-	5
41-50	5	4	-	-	-	5
51-60	3	4	-	-	-	-
61-70	3	5	-	-	-	-
71-80	3	2	-	-	-	-
≥ 81	3	-	-	-	-	1
Total	45	38	08	12	09	11

DISCUSSION:

Tissier was the first one to report a Y- shaped bacterium in infant faeces and named it as Bacillus bifidus communis, later in the genus Lactobacillus and finally assigned to a genus Bifidobacteria in 1974.[14] The genus Bifidobacterium inhabit gastrointestinal tract and helpful in maintaining human health as they possess probiotic potential according to some investigators.[3,4] In a preliminary study conducted in our institute, few strains of Bifidobacteria exhibited antagonistic activity against aerobes and certain anaerobes including enteric pathogens which has to be proved by employing more number of isolates.

Speciation of Bifidobacteria by biochemical characterization had some hurdles. Few of the biochemical reactions gave similar results for some species, hence we had to depend on Maldi Tof for the identification of species which had confusing reactions. Absence of Anaerobic culture facilities and Maldi Tof in most of the institutes will have a tough time with the speciation of Bifidobacteria.

It is stated that the factors such as mode of delivery, feeding habits, immune response immaturity, exposure to broad spectrum antibiotics have significant impact on the initial colonization of the Gastrointestinal tract and the microbial make up.[15,16] Recent studies using molecular techniques have indicated that there are differences in the composition of gut microbiota between infants, adults and elderly population.[11] In the present study, 123 Bifidobacteria species were isolated from 233 faecal specimens tested, predomoinated by B.longum which was isolated from all age groups followed by B.bifidum. However, B.bifidum was absent in the age group 0-10 and also above 80, in the present study. B.breve and B.adolescentis and B.catenulatum were found only in the paediatric population. These results were concordant with the studies conducted by Kato et.al,where they investigated the gut compositional changes of Bifidobacterium species by Real time PCR with species specific primers employing wide range of age groups. [11] According to Averishna et.al, B.breve was highly prevalent in infant gut microbiota and showed negative correlation with B.longum in the adult population.[2] In our study, B.breve was detected only in 0-10 group. Similar to the findings of Satokari et.al,[17] the proportion of Bifidobacterium decreases in the adult population.

Eventhough Bifidobacteria is not a documented pathogen, they are reported to have an invasive potential in the immunocompromised hosts. Bush etal reported the sepsis of prosthetic joint post total hip arthroplasty in a person due to *B.tertium* .[8] Its presence in the synovial fluid was attributed to the intake of probiotic formulation containing strains of Bifidobacteria. Few species of Bifidobacteria such as *B.dentium,B.denticolens, B.inopinatum* were commonly associated with dental caries. [18,19] However, in our study 11 strains of B.dentium were isolated from faeces of age groups (10 from 31-50 and 1 from above 81 age groups) and it was not possible to correlate the dental status of these cases. Bacteremia in preterm infants due to Bifidobacteria had been reported by few investigators [9,20,21] but the occurrence of sepsis by *B.longum* in adult is rarely documented. Invasive infections such as meningitis, endocarditis, peritonitis, intra-abdominal enterocolitis, gynecologic and pulmonary infections involving Bifidobacterium were also reported by various researchers.[9,10,21,22] Schwiertz et al., reported increased population of Bifidobacteria along with anaerobes such as Bacterioides spp in bacterial vaginosis cases.[23] Cases of abdominal abscesses, obstetric and gynecologic infections were also attributed to bifidobacteria by Brook and Frazier.[24] A recent article quoted that low abundance of Bifidobacteria was noticed in the lower gut microbiota in patients with H.pylori related gastric ulcer and gastric cancer.[25]

In conclusion , the distribution of various species of Bifidobacteria was seen in various age groups as reflected in this study as well as in the literature. Despite the beneficial role of Bifidobacteria, few adverse effects were also reported by certain investigators. Taking all these facts into consideration, Borriello etal recommended that newly introduced probiotic strains from commensals flora must be without any intrinsic antibiotic resistance and it is essential to be aware of the risks and benefits involved.[26]

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