

**CHARACTERIZATION OF INTESTINAL MICROBIOTA IN CELIAC CHILDREN**

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**ABSTRACT**

Celiac disease (CD) is enteropathy autoimmune induced by the ingestion of gluten in genetically predisposed subjects. The ingestion of gluten is responsible for the symptoms of CD, and a disturber of the intestinal microbiota. In this study, 13 Samples of intestinal biopsy, 15 fecal samples from celiac children, and 10 from controls children respectively were collected and analyzed by conventional culture technique to characterize the microbial profile intestinal of celiac children. There was 24 celiac children (8 boys), Mean age at diagnosis was 9.52 years, all have clinical manifestations, positive anti-transglutaminase antibodies and mucosal lesions suggestive of CD (Marsh Classification). We found a difference in intestinal microbiota, between celiac and controls children for example the *Enterobacteria*, *Clostridium sp* and *Staphylococcus sp* were remarkably higher in biopsy and fecal samples of celiac children than in controls. Inversely the *Enterococcus sp*, *Lactobacillus sp* and *Clostridium sp* were slightly lower in celiac children. Our results indicate an imbalance in intestinal microbiota for celiac children as reduction in the numbers of *Lactobacillus sp*, *Enterococcus sp* and increases in the numbers of *Enterobacteria*, *Staphylococcus sp* and *Clostridium sp*.

**Keywords:** Celiac disease, Intestinal Microbiota, Anti-transglutaminase, *Lacobacillus sp*.

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## 1. INTRODUCTION

Celiac disease is enteropathy autoimmune induced by the ingestion of gluten in genetically predisposed subjects [1]. Gluten is a protein found in wheat and other cereals (hordein, rye) [2,3]. This disease can manifest at any age and present a variety of clinical symptoms [4]. The most typical symptoms are classical gastrointestinal tract (GT) complaints [5]. Atypical celiac disease is usually seen in older children and characteristics of malabsorption are absent. Symptoms can be intestinal or extra-intestinal [6, 7]. The apparition clinical signs of this disease are caused by several factors among, the ingestion of gluten, and it has been suggested to be associated with the imbalance of intestinal microbiota [8, 9, 10].

The microflora of our gastrointestinal tract, called intestinal microbiota was estimated at about  $10^{13}$ - $10^{14}$  microbial cells, within this intestinal community, about 400 to 1000 different bacterial species were recorded [11]. These bacteria account for 40% of the fecal weight [12]. Microbial colonization intervenes at the time of birth, where newborn colonizes primarily from the vaginal and fecal flora maternal [13]. During the first 12-24 hours of life the first colonizing bacteria appear in his digestive tract, are *Escherichia coli* and *Enterococcus sp*, then strictly anaerobic strains appear faster such as *Bifidobacterium sp* along with some *Bacteroides sp*, if the newborn is fed to the breast and for children fed with infant formulas, the *Bacteroides sp* is predominant [14,15]. The composition of intestinal microflora is similar to that of adults around the age of 2 years when the child gradually shifts of a diversified diet [16].

The balance of intestinal flora involved to regulate the intestinal transit and to prevent the proliferation, of pathogens [17]. That balance is disturbed by various factors as stress, taking antibiotic an unbalanced diet regime, and as disease for example celiac disease [18,19].

The aim of the present study was to characterize the flora starting at biopsy and fecal samples in celiac children and controls children by microbiological analysis to study the intestinal microbial profile of celiac children.

## 2. MATERIALS AND METHODS

### 2.1. Sampling

The 13 duodenal biopsy samples and 25 fecal samples were collected from two groups of children; (1) group of celiac children and (2) group of controls between April 2013 to June 2015.

### 2.2. Inclusion criteria

Children included in this study:

- Having only celiac disease.
- Without following treatment with antibiotics or corticoids at least one month before the period of sampling.
- The children were enlisted in the study after informed consent was achieved by their parents and the Ethics Committee of the faculty of Medicine - Oran- Algeria.

### **2.3. Serological analysis**

Serological screening of celiac disease was carried out on a blood sample obtained from each patient; samples were separated from by centrifugation. Detection of anti-Tissue transglutaminase (IgA and IgG) in serum was performed by fluoro-enzyme-immuno-assay (FEIA) using a commercial kit, Test EliA Celikey IgA (Thermo Fisher Scientific) [20] or by enzyme-linked immunosorbent assay (ELISA) technique (positive result > 10 U/ml).

### **2.4. Histological analysis**

Biopsies sample were taken during upper endoscopy (Pantax FG 24W) from the second or third portion of duodenum (at least four biopsies). Each biopsy was laid on filter lame, fixed in 10% formalin, and evaluated by an expert gastrointestinal pathologist. Histopathological finding were staged according to the Marsh classification [21].

### **2.5. Microbiologic analysis**

#### **2.5.1. Samples preparation**

Duodenal biopsy and fecal samples were collected in sterile recipients, approximately 10- 15 mg of each duodenal biopsy, and 1g weight for each fecal sample were diluted in 9ml sterile physiological water (9 g sodium chloride, 1000 ml distilled water and pH= 07) for obtained dilution ( $10^{-1}$ ), decimal dilutions were performed up to ( $10^{-9}$ ), homogenized by thorough agitation in a vortex and 100  $\mu$ l of each dilution was spread on different selective agar media in duplicated.

Total Aerobic Mesophilic was isolated on Nutrient agar, *Enterobacteria* were isolated on Eosin Methylene Blue agar (EMB agar), *Enterococcus sp* were isolated on Bile Esculin agar (BEA agar), *Staphylococcus sp* on Chapman agar. All culture media were incubated in aerobic conditions at 37 ° C for 48 h.

Total Anaerobic Mesophilic was isolated on Nutrient agar, *Lactobacillus sp* were isolated on Man, Rogosa and Sharpe agar (MRS agar), *Bifidobacterium* on MRS agar supplemented with 0,05 % cysteine and *Clostridium* on Liver Meat agar supplemented with 0.5 ml of sodium sulfite and some drops of iron alum, after incubation in anaerobic conditions at 37°C for 72 h.

*Yeast* was isolated on Sabouraud Chloramphenicol agar and incubated at 30-37°C for 3- 5 days.

After incubation, different colony types were counted. The enumeration of germs was made on the dishes presenting between 30 and 300 colonies and expressed in log colony forming units (CFU) per gram of fecal sample, by the following formula [22]

$$\text{Log CFU/g} = \text{Log} \frac{\text{Number of Colony}}{\text{Dilution X volume seeded}}$$

The identification of colonies found in each culture medium was confirmed by macroscopic examination, the Gram stain and the use of biochemical tests [23].

## 2.6. Statistical analysis

The results (age, Weight, Height, Body Mass Index and t-TG) are presented as mean and Standard deviation (SD). The difference in microbial groups between controls and celiac children were determined by Colony Forming Unit (CFU).

## 3. RESULTS AND DISCUSSION

### 3.1. Clinical Characteristics

The clinical, serological and histological evaluations for two groups included in the study are summarized in **Table 1**. The group celiac Children at mean age 9, 52 years, with 16 girls, the group controls children at mean age 9,4 years with equivalent number of female and male and for the median of Body Mass Index (BMI) was lower in children with celiac disease compared to control children (median 14.82 kg/m<sup>2</sup> and 16.25 kg/m<sup>2</sup> respectively).

The lesions of duodenal biopsies were observed in our celiac children, but with grade different according to the Marsh classification, and all patients' children had positive t-TGA antibodies at mean concentration 113 UI/ml. The serological and histological analysis with the clinical symptoms observed at the time of diagnosis confirms the presence of celiac disease in these children.

We observed, The BMI of most celiac children corresponds to a normal weight but remains close to underweight area that can linked to malabsorption of nutrients in the small intestine.

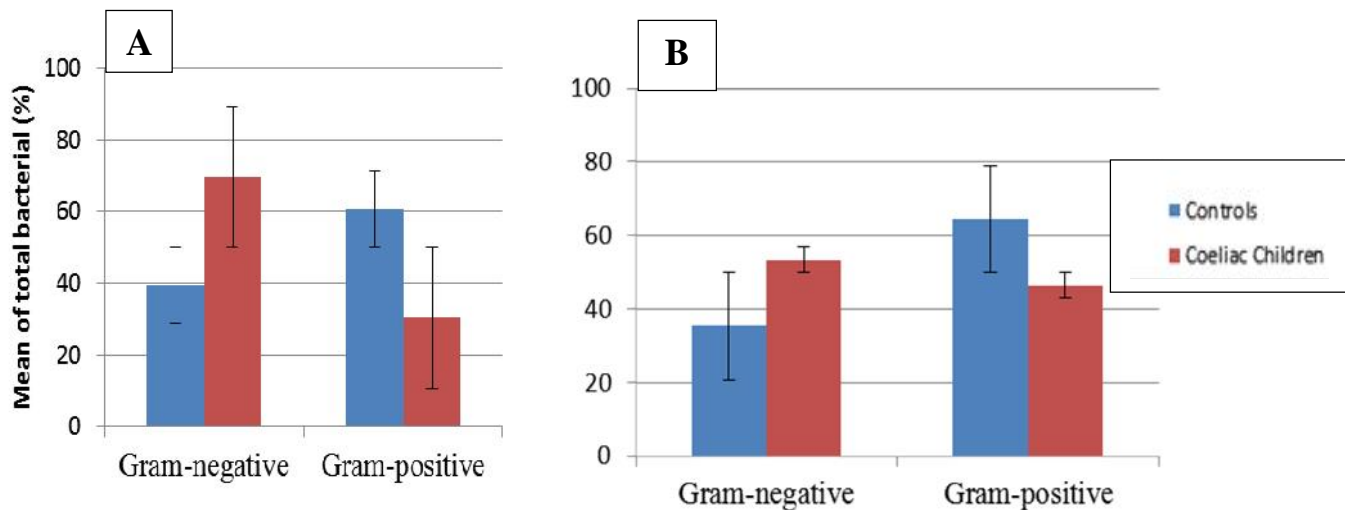
**Table1.** Clinical, serological and histological characteristics of children

<b>Characteristics</b>	<b>Celiac children n=24</b>	<b>Control children n= 10</b>
Age (years)	9,52 ±3,66	9,4 ±2,22
Gender		
- Girls	16/ 24	5/10
Weight (kg)	24.34 ± 9.53	30,4 ± 7.27
Height (m)	1,26 ±0,18	1,36± 0.14
Body Mass Index( kg/m <sup>2</sup> )	14.82 ± 1.87	16.25 ± 1.43
<b>Clinical Symptoms</b>		
-Abdominal pain	16/24	-
-Staturo-ponderal delay	13 /24	-
-Wasting	1 /24	-
- Diarrhea	11/24	-
- Vomiting	6/24	-
- Anorexia	4/24	-
-Constipation	4/24	-
-Anemia	3/24	-
-Dental enamel defects	3/24	-
<b>Serology</b>		
-t-TG =anti-transglutaminase (UI/ml)	t-TG+ (113)	-
<b>Histology</b>		
-Garde I	0/24 (0%)	-
-Garde II	9/24(37.5%)	-
-Garde III	15/24(62.5%)	-

(-) undetermined

### 3.2. Microbiological analysis

The total number of gram negative bacteria was high in the fecal matter and biopsy samples of celiac children than controls and against the total number of gram-positive bacteria has been decreased in celiac when compared to control (**Figure 1**). That result obtained is shown also by Nadal *et al* [24].



**Fig.1.** General composition of intestinal microbiota of coeliac and control children, (A) Duodenal microbiota, (B) fecal microbiota

In this work we analyzed the duodenal biopsy and feces of celiac children and controls by classical microbiological techniques **Figure 2** and **Figure 3**.

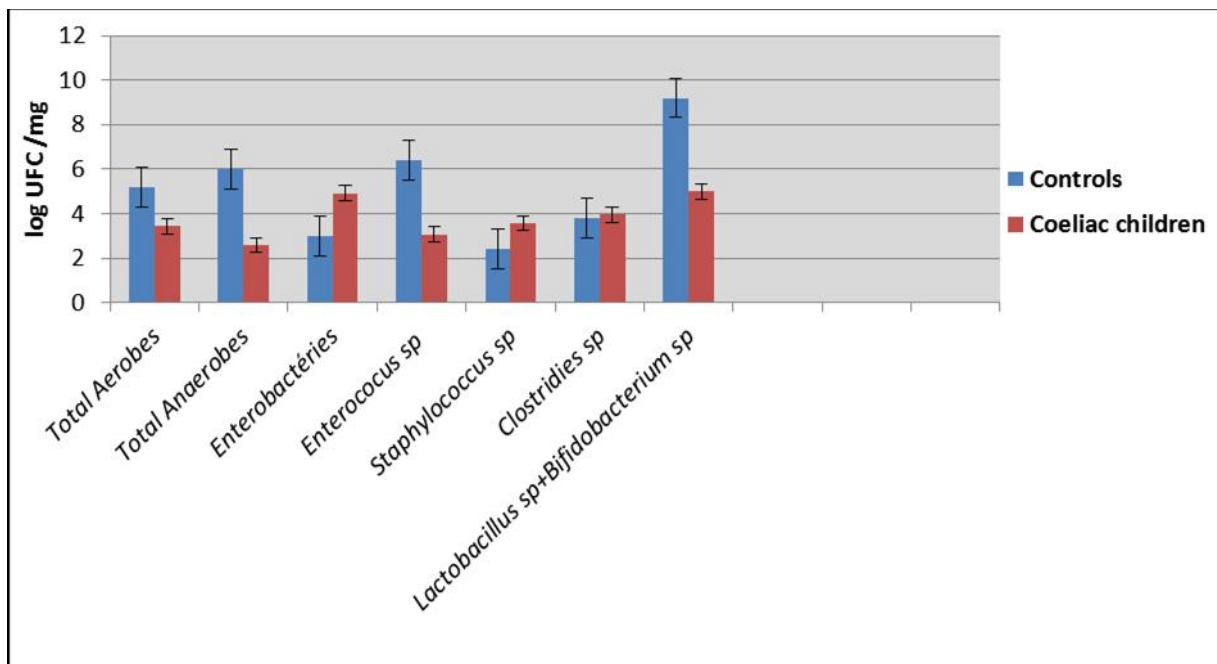
The highest differences detected for the different microbial groups in celiac and controls children; as the *Enterobacteriaceae*, *Staphylococcus sp* and *Clostridium sp* were more abundant in duodenal sample of celiac children with median of 4.92 log CFU/mg, 3.56 CFU/ mg and 3.95 log CFU/mg respectively than in controls with medium of 3.00 log CFU/mg, 2.4 CFU/mg and 3.80 log CFU/mg.

Also for fecal sample, these previous groups were higher in celiac children with medium of 7.60 log CFU/g, 4.05 log CFU/g and 2.81 log CFU/g at celiac children respectively than in controls with medium of 6.81 log CFU/g, 2.81 log CFU/g and 1.76 log CFU/g.

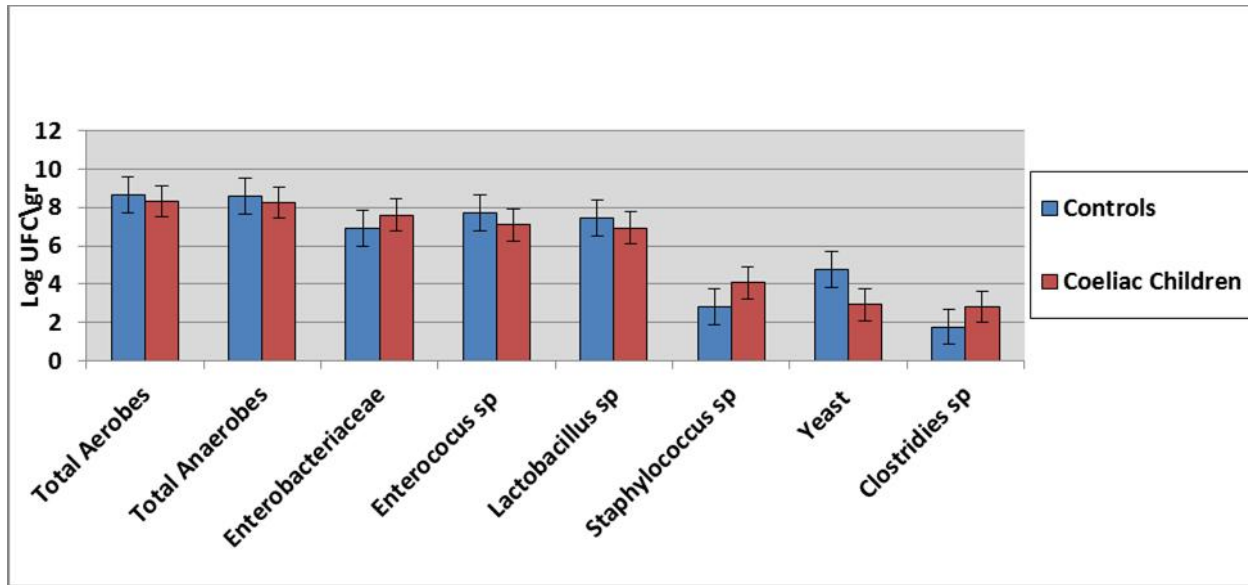
Our results shows that celiac children had a higher abundance of *Clostridium sp* in duodenal and fecal microbiota, these findings were the same as reported in study of Collado *et al* [25,]. The increase in the concentration of *Enterobacteriaceae* cells have been detected in agreement with a previous study of fecal collected only from celiac patients [25, 26, and 27]. In addition the increasing number of *Staphylococcus sp* in feces from celiac children can be a consequence of inflammation of the intestinal mucosa owed by the ingestion of gluten [28]. Increased levels of *Staphylococcus sp* and *Enterobacteriaceae* were also higher in allergy infants compared to healthy infants, suggesting a relationship between the bacterial group and immune dysregulation [28].

The total of the *Lactobacillus sp* plus *Bifidobacterium sp* populations were shown to be different in duodenal sample of CD patients and control children with median value 5.00 log CFU/mg and 9.2 log CFU/mg respectively. But, the numbers of median for the *Lactobacillus sp* and *Yeast* are less abundant in fecal of celiac children (6.92 log CFU/g, 2.92 log CFU/g for) than in controls (median= 7.43 log CFU/g, 4.73 log CFU/g) and for the *Enterococcus sp* was abundant in control children than in celiac children.

We found, the lower numbers of *Lactobacillus sp* strains in celiac children who agreed with other reports [30, 31]. The compositions of the *Lactobacillus sp* and *Bifidobacterium sp* populations were shown to be different in celiac disease patients and healthy children. It is known that the *Lactobacillus sp* and *Bifidobacterium sp* are probiotic strains help to keep health and restoration of intestinal biotope [32].



**Fig.2.** Duodenal microbiota composition of celiac children and controls, determined by using selective culture media,



**Fig.3.** Faecal microbiota composition of celiac children and controls, determined by using selective culture media

The modifications sensed in the composition of the fecal microbiota from the celiac disease children may be the cause of the disease. [24].

#### 4. CONCLUSION

The results of this work demonstrate a difference in the microbial groups of duodenal and fecal microbiota from celiac children when compared with controls. The diversity of the fecal microbiota in celiac children is characterized by a reduction in the numbers of *Lactobacillus sp*, *Enterococcus sp* and increases in the numbers of *Enterobacteria sp*, and *Staphylococcus sp*. We can say that celiac disease is considered a factor causing an imbalance of the composition of the intestinal microbiota.

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