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Characterization of *Enterobacteries* Genus in Intestinal Microbiota of Celiac Children

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Abstract The imbalance of the intestinal microbiota is link, by several diseases such as celiac disease, which causes inflammation in the small intestine. This inflammation is due by the digestion of gluten present in some type of cereals. In this work, fecal and duodenal biopsy samples were collected to characterize by conventional culture technique the composition of the Enterobacteries group in intestinal mirobiota of celiac children and were compared with control children. A significant difference detected in the intestinal flora of celiac children compared to controls children concerning the *Enterobacteries* group. We found an increase of *E.coli*, *Enterobacter aerogeneses*, and *Klebsiella* with presence of *Salmonella sp*, *Shigella sp* in biopsy and fecal samples of celiac children and a relationship between the increase of *Enterobacter cloacea* and the presence of positive anti-transglutaminase value.

Keywords: *celiac disease, intestinal microbiota, enterobacteries, E.coli, anti-transglutaminase*

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1. Introduction

Intestinal flora is a complex microbial consortium. It participates actively in our good health. [1,2]. This microbial population hosted in the human digestive tract and contains approximately 10^{14} bacteria [3]. The intestinal microbiota is considered as a full organ which plays an important role in energy metabolism, and nutritional balance [4]. It also serves to stimulate the intestinal immune system and opposes the intestinal colonization by pathogenic microorganisms (barrier effect) [5,6]. These functions can be influence if the composition of the flora is changed. Changing the composition of the intestinal flora both, quantitative and qualitative level may be the cause of intestinal disorders like diarrhea or chronic inflammatory diseases such as celiac disease [7].

Celiac disease (CD) is an autoimmune affection that occurs in genetically susceptible individuals [8]. This disorder results in damage to the wall of the small intestine mucosa. When foods with gluten consuming. Gluten is a form of protein found in some cereals as (wheat, (hordein and rye) [9,10]. The damage to the intestine mucosa leads to poor absorption of many nutrient specially (fat, calcium, iron, and folate) Which results in a growth retardation in children and a risk of osteoporosis in adults [11,12] and also causes in change of the bacterial groups of intestinal flora as Enterobacterie [13].

The objective of this study was to determine the composition of Enterobactries group starting at biopsy and fecal samples in celiac children and controls children by classical microbiology technical.

2. Materials and Methods

2.1. Subjects and Samples Collection

The duodenal biopsy samples and fecal samples were collected from two groups of children; (1) group of celiac children, having clinical manifestations evoking the celiac disease, occurring after the introduction of gluten in the diet with a confirmation of the disease by histological analysis according to the classification of Marsh for different types of villous atrophy and serological analysis, such children have no other pathology. For (2) group of controls, are non-celiac children, have a normal villous structure and a negative serology. The two groups of children do not follow any treatment with antibiotics or corticosteroids at least two month prior to sampling period. A total 30 fecal and 19 duodenal biopsy of these children were collected in sterile jars labeled with the name of the child.

2.2. Ethical Consideration

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. Sample Treatment

One gram of each fecal sample and per milligram of intestinal biopsy (grinding using a scalpel) were weighed and placed into 9 ml of sterile physiological water; it is the

mother solution, then decimal dilutions were performed up to (10^{-8}). 100 µl of each dilution was spread on Eosin Methylene Blue agar media (EMB agar) and on Hektoen agar in duplicated. All culture media were incubated in aerobic conditions at 37°C for 48-72 h.

2.4. Bacterial Enumeration

After incubation, Enumeration of germs was conducted on the dishes presenting between 30 and 300 colonies and expressed in log colony forming units (CFU) per gram for statistical reasons by the following formula [14]:

$$\text{Log CFU / g} = \text{Log}\frac{\text{Number of Colony}}{\text{X dilution}}.$$

2.5. Identification of the Bacterial Strains

2.5.1. Morphological Characters

The macroscopic observation of the colonies used to show some specific characteristics of species such as: appearance, size, contour and pigmentation.

To verify the purity of the isolates using the Gram stain. This allows to make the differentiation between Gram-positive and Gram-negative bacteria and thus to describe the form of bacterial cells and their association mode.

2.5.2. Biochemical Characters

The study of biochemical tests is essentially based on research oxidase, catalase and urease test, B galactosidase (ONPG),Triple Sugar Iron Agar, Simmons Citrate, Indole production, Voges-Proskauer (VP), Methyl red test, and after these classical methods [15], bacterial strains were confirmed by using the kits commerciels API 20E.

2.6. Statistical Analysis

The results achieved were expressed as mean and standard deviation ($x \pm \delta$) and for the microbiological analysis of the intestinal microbiota for two groups of children were determined by Colony Forming Unit Log (CFU) and value of $p \leq 0.05$was considered significant.

3. Results and Discussion

3.1. Subjects

45 Children enrolled in this work with a mean age of (9.62 ±0.90years), celiac children n =24, with 08 boys and control (no celiac children) n=21 with 10 boys. Form the mean of Body Mass Index (BMI) was somewhat weak in children with celiac disease than control children (median 14.82 kg/m^2 and 16.26 kg/m^2 respectively).This value of BMI in celiac children may be associated with malabsorption of nutrients in the small intestine that is characterize by villous atrophy.

3.2. Enumeration of *Enterobacterie* Group in the Intestinal Microbiota

Figure 1 indicates that the median of *Enterobacteries* found in fecal sample (7.60 Log CFU) of celiac children was higher when compared with control children (6.25 Log CFU). Also for duodenal sample, the *Enterobacteries* genus was more abundant in celiac children with medium of 4.92 Log UFC than in controls with median of 3 Log UFC. Our results obtained were similar with other studies that have examined the fecal matter of celiac children [16,17,18]. As the study of Björkstén *and al* was indicated the increase number of Entrobacteries in allergy infants compared to healthy infants suggesting a link between the bacterial group and immune dysregulation [19].

3.3. Biochemical Identification of Enterobacterie Genera

Enterobacteries strains isolated from the EMB agar media were presented different form like dark violet colonies, domedshowa greenish metallic sheen light or blue colonies with dark brown center, flattened and sometimes-brownish colonies, mucosal. Microscopic examination by Gram coloration shows that our strains are gram-negative bacilli and the oxidase test was negative in all cells bacterial. After biochemical tests, the strains of *Enterobacteries* identified in the intestinal flora of celiac children are: *E.coli, Enterobacter aerogeneses, Enterobacter cloacae, Klebsiella oxytoca, Salmonella sp, Citrobacter sp, Shigella sp.*

3.4. Composition and Diversity of *Enterobacteries* genus in Intestinal Flora of Celiac Children

From the results shown in Figure 2 and Figure 2, we detected a difference for *Enterobacteries* genus in the intestinal (fecal and duodenal) flora of the celiac children compared to the controls. This difference was showed by the increase in the number of the *E.coli* and *Enterobacter aerogeneses* from the duodenal flora of the celiac children with medium (3.99 Log CFU/mg, 1.32 Log CFU/mg respectively) compared to the control with medium (2.43 Log CFU/mg, 0.4 Log CFU/mg)**.** Same result obtained for fecal matter, these genera were slightly higher in celiac children with medium (4.33 Log CFU/g , 1.74 Log CFU/g respectively) than control with medium (4.05 Log CFU/g ,1.35 Log CFU/g).

We found also the genera of *Enterobacter cloacae* and *Citrobacter* only in the intestinal biopsy of the celiac children with a very high medium (1.00Log CFU/; 0.47 Log FCU/mg respectively) compared to the control (0.13Log CFU / mg; 0.04Log CFU/mg).

And for *Salmonella sp, Shigellasp* and *Klebsiella sp* were identified only in the intestinal microbiota of celiac children with different values (0.60 Log CFU / g; 0.48 Log CFU / g and 0.15 Log CFU / mg), and slightly higher rate of *Klebsiella oxytoca* was recorded in fecal samples of coeliac children compared to controls (0.81LogCFU / g, 0.18 Log CFU / g). The abundance of *E. coli* in celiac children compared to controls was reported by the study of Schippa *et al* [20] also at adult and children in Crohn's disease [21].

In addition, *Escherichia coli* adherent invasive able to replicate and induce tumor necrosis factor production (TNF-α) in the intestinal mucosa of patients with Crohn's disease [22].

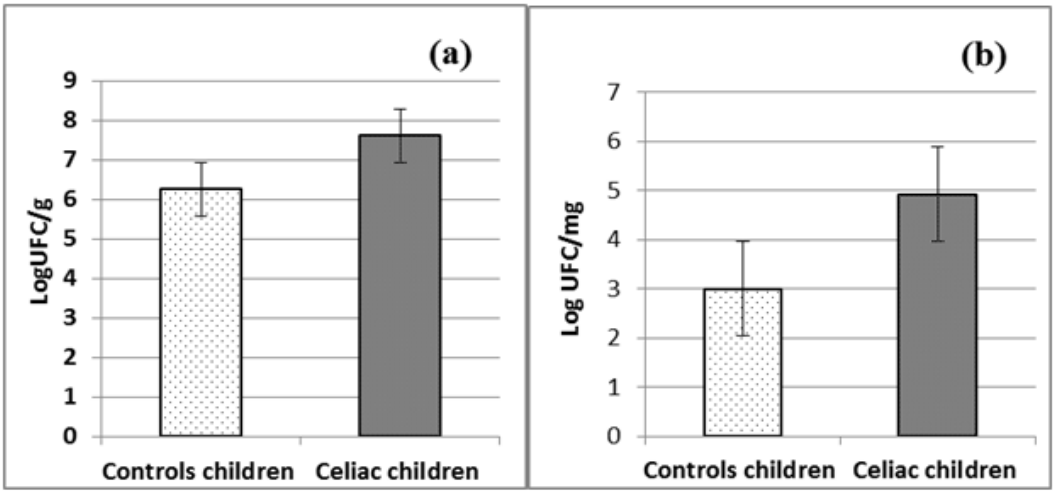

Figure 1. Mean of *Enterobacterie* genera in fecal microbiota (a) and duodenal microbiota (b) of controls and celiac children

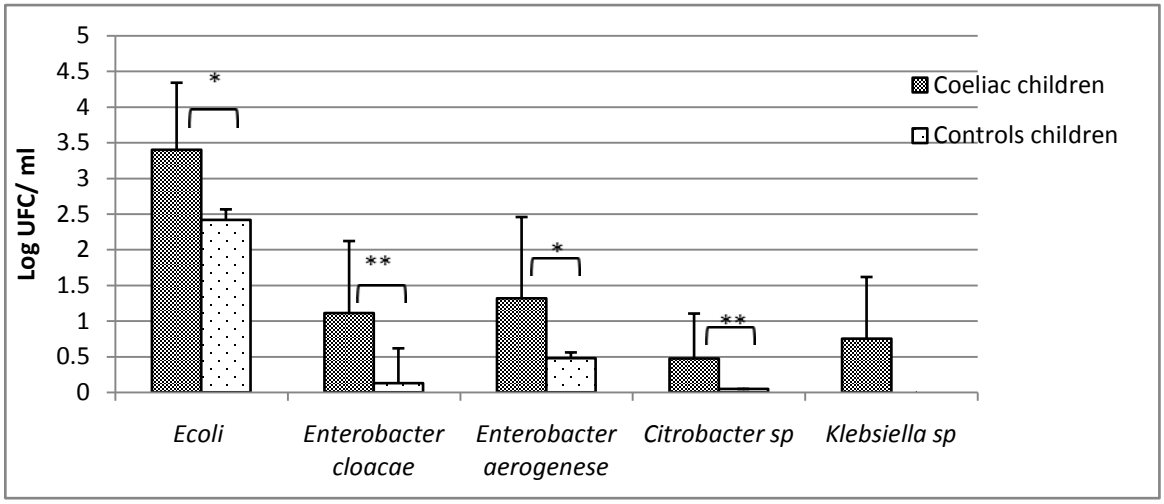

Figure 2. Different genus of *Enterobacteries* fond in the duodenal microbiota of celiac and controls children (*: P<0, 05, ** : P<0,01)

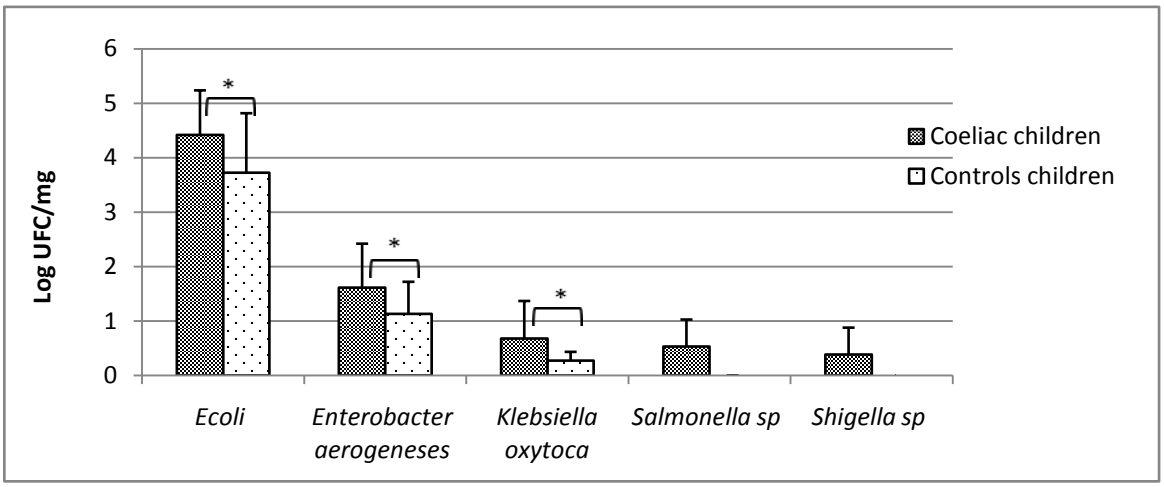

Figure 3. Different genus of *Enterobacteries* found in the faecal microbiota of celiac and controls children (*: P<0,05)

Generally, *E. col*i was the most common species of the *Enterobacteriaceae* family detected and isolated from stool samples and intestinal biopsies of celiac children [23,24].

Our results are in agreement with the work of Di Cagno *et al* [25], which showed that *Salmonella*, *Shigella* and *Klebsiella* were significantly higher in celiac children. The studies of Sánchez *and al* [18,26] have revealed the abundance of family *Enterobacteriaceae* in celiac children, particularly *Klebsiella oxytoca* strains, *E. coli* and *Enterobacter cloacae*. *Enterobacteria* of the *genus Klebsiella*, *Enterobacter*, and *Citrobacte* present the passage flora by concentrations below 10^6 CFU / g, and colonize the digestive tract, during a pathological situation [27].

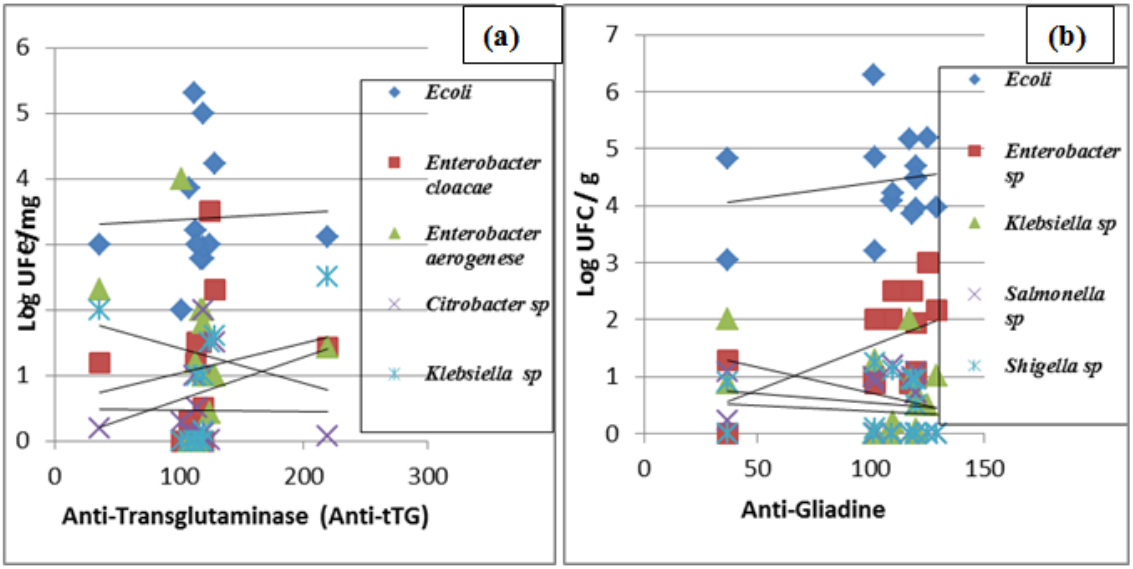

Figure 4. Correlation between anti-Transglutaminase (Anti-tTG) and of *Enterobacteries* group in celiac children (a) intestinal biopsy and (b) fecal matter

3.5. Correlation between Anti-Transglutaminase (Anti-tTG) and Genera of *Enterobacteries* Composition in Celiac children

A significant correlation was observed only between the number of *Enterbacter cloacea* (r = 0.59), and the level of anti-transglutaminase antibody in intestinal biopsies and feces in celiac children (P < 0,05).

Negative correlations were recorded between the genera of *Enterobacteria* and anti-gliadin antibody levels in celiac children regardless of the site of biopsy or fecal sampling.

Our study shows positive correlations only between the serological markers anti-tarnsglutaminase antibodies and the numbers of *Enterbacter cloacea*. This suggests that, the increase in *Enterobacter cloacea* is related to the positive anti-transglutaminase values in the small intestine in celiac children. No results published so far, for this context, but various studies have demonstrated associations between the disturbance of the intestinal microbiota, and the pathophysiology of celiac disease. Nevertheless, the alteration of the intestinal microbiota is not yet elucidated, if it is the cause or consequence of the celiac disease. Verdu *et al* [28] have shown that genetically predisposed individuals may exhibit an imbalance of the intestinal microbiota, increasing the immunological responses responsible for intestinal lesions, which would promote the onset of celiac disease. Other studies suggest that auto-antibodies such as anti-transglutaminase antibodies may also modulate haemostasis of the small intestine, the onset of villous atrophy, which alter the permeability of the intestinal barrier and facilitate the passage of bacterial pathogens to colonize the intestinal mucosa [29,30].

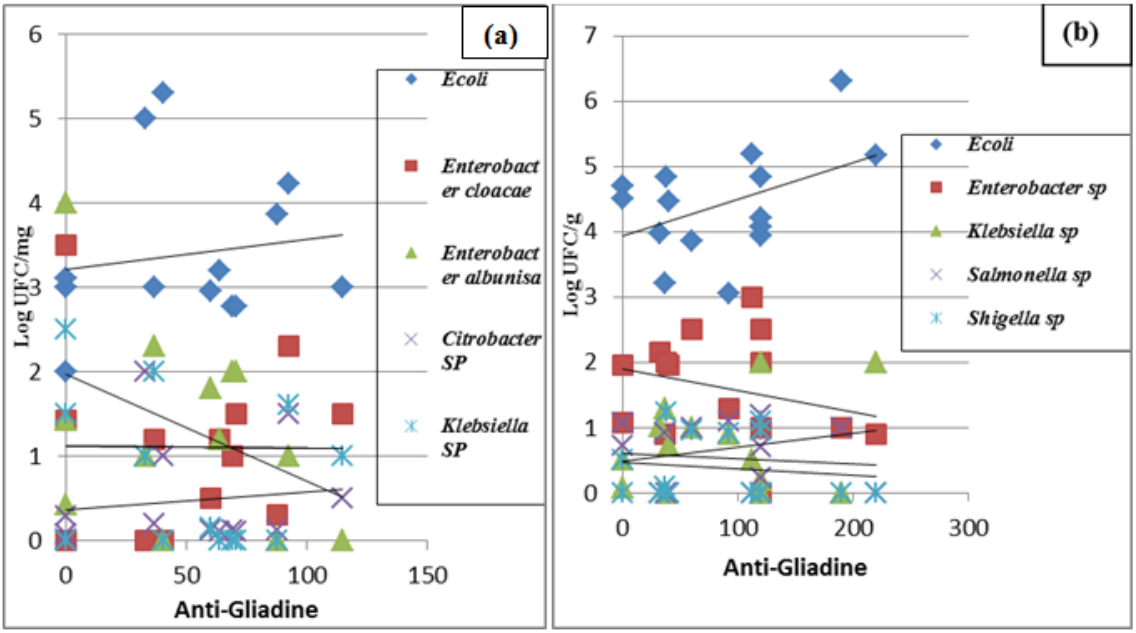

Figure 5. Correlation between anti-Gliadine and of *Enterobacteries* group in celiac children (a) intestinal biopsy and (b) fecal matter

4. Conclusion

Our work demonstrates a quantitative and qualitative diversity in the group of *Enterobacteriaceae* of the duodenal and fecal microbiota from celiac children compared to the controls. This difference is detected by the increase in the number of *E.coli*, *Enterobacter aerogeneses, Klebsiella sp* and the presence of *salmonella sp, Shigella sp in* celiac children more the increase in *Enterobacter cloacea* is related to the positive anti-transglutaminase values in the small intestine in celiac children. Therefore, the results show that the imbalance of this bacterial group in the intestinal microbiota can increase the pathogenesis of celiac disease.

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