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ORIGINAL ARTICLE

Crohn's Disease: Retrospective Study In Algerian Patients

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ABSTRACT

Background: Inflammatory disease of Crohn affects the entire digestive tract, with extra-intestinal manifestations and immune disorders.

Objectives: This work aims to represent the histopathological aspects of Crohn's disease and the establishment of pathogenic bacteria as causal agents.

Methodology: The histopathological aspects of the disease were studied on a colonic resection specimen and on intestinal biopsies with colorations topographic staining. Pathogenic bacteria responsible for Crohn's disease have also been isolated and identified. The study is continued to establish a correlation between the disease and exposure to infections by unusual bacteria, particularly pathogens (*Salmonella, Shigella, Streptococcus pyogenes, Klebsiella*).

Results : The macroscopic appearance of the disease presented transmural involvement and can be complicated by abscesses and fistulas, microscopic appearance indicated the infiltrate of inflammatory cells (lymphocytes, plasma and cells) and lymphoid follicles after topographic staining.

Crohn's disease is an idiopathic disease, it is assumed that it is a deregulation of the immune system due to an infectious agent in genetically predisposed people.

In our work, we studied the microbiota of intestine and stool of Crohn's patients in which we found certain bacteria including *Proteus mirabilis* with a predominance of *E. coli.* Other pathogenic bacteria were found like *Salmonella* spp., *Shigella* spp., *Klebsiella* spp. and *Streptococcus pyogenes*. Two cases which tested positive on Ziehl Neelsen stain represented *Mycobacterium avium paratuberculosis*.

Conclusion: The histopathological aspect of CD can be better visualized and identified on surgical specimens than on endoscopic biopsies, which helps to monitor the evolution of the disease and must be accompanied by clinical, serological and radiological exploration.

Keywords
Crohn's disease, inflammatory granulomas, lymphoid follicle, Ziehl Neelsen staining, Hematoxylin eosin staining, Mycobacterium avium paratuberculosis.

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INTRODUCTION

Crohn's disease is IBD[1], affects the entire digestive tract, most often ileum and colon[2]. It's involvement is transmural that is mucosal involvement extending to all layers of the wall. It's diagnosis is based on a range of clinical, biological, endoscopic and histological arguments[3]. It develops in flare-ups, interspersed with remissions, and

can be complicated by structures, abscesses and fistulas. The goal of treatment is to heal the lesions before the occurrence of irreversible complications[3].

Crohn's disease has two main peaks of occurrence in humans, the first around the age of 30 and second peak around the age of 60-70. However, it can occur at any age, especially in children and the elderly. Crohn's disease mainly affects women with a sex ratio of 1.2. Every 6 to 8 patients per 100,000 inhabitants in France are affected with Crohn's disease which corresponds to a prevalence of 80,000 cases in France. This incidence is highly variable depending on the region with a North-South gradient found between the countries of Northern Europe and those of the South[4].

Theories of CD etiology with many factors were proposed: alimentation, environment also dysregulation against the microbiota, all of the work have been viewed regarding genetic predisposition of patients[5]. The objectives of this work are to present the histopathological aspects of CD on a colon resection specimen and on intestinal biopsies with HE topographic staining, to identify the pathogenic bacteria as causative agent (*Mycobacterium avium paratuberculosis*, *E. coli*) and to determine the correlation between the disease and exposure to infections by unusual bacteria, particularly pathogens (*Salmonella*, *Shigella*, *Streptococcus pyogenes*, *Klebsiella*).

MATERIALS AND METHODS

Workplace

Our experimentation was performed in several units lasting four months from February to May 2015:

- Gastroenterology Department- Mustapha Bacha (Algiers); Internal Medicine Department-UHC Kouba (Algiers); Internal Medicine Department-UHC Beni Messouse (Algiers) for the collection of intestinal biopsies (two fragments in 10% formalin and two others in physiological water).
- Anatomopathological and cytological department-UHC (Medea) for the anatomo-pathological examination of intestinal biopsies and of the colonic resection specimen.
- Ain Defla medical analysis laboratory of Dr Zibouche for the bacteriological examination of intestinal biopsies.

Biological material

Intestinal biopsies were obtained from the 15 patients representing ileal and colonic CD (diagnosed) who underwent low endoscopy (control colonoscopy), for the search for MAP and bacteriological examination; two fragments were placed in physiological saline to store at 4°C. Biopsies should be taken from pathological and healthy mucosa for bacteriological examination; and two biopsies fragments were taken from pathological mucosa embedded in 10% formalin for pathological examination (Table **1**).

Table 1. Medical characteristics of patients.

Number	Sex	Age (years)	Duration of illness (years)	Diagnosis/ localization CD	Surgery
01	Male	32	4	ileo-colic	No
01	Female	41	18	diffuse, several locations	Partial colectomy (distal part of right colon)
02	Male	39	6	ileal	No
02	Male	25	3	Colic	No
02	Female	17	0.8	ileal	No
02	Female	22	3	ileocecal	No
02	Female	53	14	ilea	No
01	Female	23	3	Colic	No
01	Female	23	2	Colic	No
01	Female	23	4	Colic	No

Stools from the same patients (5 out of 15 patients) were collected in sterile plastic pots for comparison of bacteriological results.

An operating specimen of an old woman of 39 years. undergoing a colectomy in the Medea (internal medicine department), presented a treatment failure for a month with severe colitis and stenoses explored by colonoscopy. The anatomo-pathological examination of the specimen aims to compare the histological aspects of the colonic biopsies and of the surgical specimen.

Anatomo-pathological examination

Intestinal biopsies taken from patients by lower digestive endoscopy (colonoscopy) and an operating specimen were placed in 10% formalin for anatomo-pathological examination[6]. The samples were stained by Ziehl Neelsen and HE Fast.

Bacteriological examination of intestinal biopsies

The biopsies were ground using a mortar in 0.5ml of BHIB[7]. A drop of suspension was observed between slide and coverslip to visualize the possible presence of bacteria and their mobility.

Using the loop, a drop of suspension was placed on the slide. After fixation, the smear was stained with Gram and read with the 100 immersion oil objective. Stained smear was examined to observe the bacterial diversity in the biopsy suspension.

The slides stained by the Ziehl Neelsen method from the biopsy suspension were observed under an optical microscope (bacterioscopy)[8].

Culture

A drop of the previously enriched medium was inoculated onto the Hektoen and Macconkey media using the streak method. Another drop was placed in the BGB. Incubation was done at 37°C for 24 h.

Each bacterium had a type of colony characterized by color, outline, smell, size and appearance. Cloudiness present in the BGB tube indicated bacterial growth of streptococci.

The suspected colonies were isolated using a Pasteur pipette and transferred to a sterile tube filled with 5ml of physiological water, then inoculated on Hektoen for 24 hours at 37°C. For BGB, a drop was taken using the loop and inoculated onto the blood agar medium to detect the type of haemolysis.

Identification

Macroscopic identification was performed according to the type of colonies one can suspect the bacterial species. For microscopic identification, a smear was prepared and stained according to Gram technique for each colony.

Isolates were identified by macroscopic, microscopic and biochemical characters at the API 20 E.

Table 2. Antibiotics to be tested for Enterobacteriaceae and their mode of action.

Families	Mode of action	Antibiotic
Aminosides/ aminoglycosides	Inhibition of protein synthesis	AK (Amikacin)
		CN (Gentamicin)
β-lactam + β-lactamase inhibitor	Act on the synthesis of peptidoglycan (Inhibition of bacterial wall synthesis)	AMC (Amoxicillin + clavulanic acid)
		AX (amoxicillin)
Cephalosporin	Inhibition of bacterial wall synthesis	CTX (Cefotaxim)
		KZ (Cefazolin)
		CFM (Cefixime)
Quinolones	DNA synthesis inhibitor	CIP (Ciprofloxacin)
		NA (Nalidixic acid)
Sulfonamides and their associates	Act on the synthesis of folate, puric acid and nucleic acid (DNA synthesis inhibitor)	STX (Trimethoprim + sulfamethoxazole)
Nitrofurans	Act directly on DNA causing various lesions	F300 (Nitrofurantoin)

Antibiogramme

Antibiotics to test for Enterobacteriaceae are presented in the Table**2**[9].

The diameter of the zones of inhibition was measured, and classified the bacteria into different categories including Susceptible, Intermediate or Resistant.

Coproculture

The stools were collected from the emissions in a clean container. The samples were immediately stored at 4°C in order to avoid desiccation and the proliferation of commensal bacteria and yeasts.

Any coproculture must systematically implement the search for *Salmonella* and *Shigella*. In addition to a selective isolation medium (Hektoen), an enrichment medium for *Salmonella* is essential.

The identification of pathogenic bacteria was done according to their macroscopic appearance on the agar, and biochemical identification.

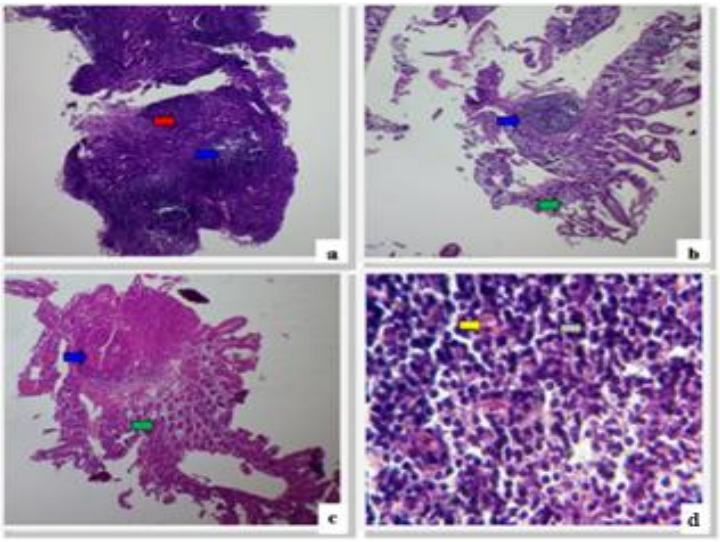
RESULTS

Histological examination of intestinal biopsies

A. After hematoxylin eosin staining

The figures of the histological sections of the intestinal biopsies are presented in Fig.**1**, in which we observe:

- Healthy mucosa with inflamed areas.
- Sub mucosa sometimes edematous.
- Reorganization of the glands can be important.
- Presence of inflammatory granuloma.
- Presence of clear centered lymphoid follicles.
- Type of ulceration: cobblestone/pebble.



Figue 1. Histological sections of intestinal biopsies.

a: HE stained biopsy section seen at low magnification (40). The fragment presents fissure ulcerations in the rotation, replacing the entire thickness of the mucosa by a polymorphic inflammatory granuloma (red arrow) arriving at the depth of the chorion with chronic infiltrate producing hyperplastic nodules presenting the lymphoid follicle with a clear center (blue arrow).

b: HE stained ileal biopsy section seen at low magnification (40). The layer of the glands are thick and of variable disposition and sometimes nibbled by inflammatory infiltrate in the acute phase (green arrow) in the center we note the presence of lymphoid follicles (blue arrow).

c: HE stained ileal biopsy section seen at low magnification (40). The fragment shows ulcerations of the epithelium with chronic inflammatory infiltrate; the glands are moderately inflammatory with a preserved appearance (green arrow) the polymorphic inflammatory granuloma and lymphoid follicle with a clear center are still present (blue arrow).

d: At the magnification 100, the level of the follicle represents a richness in polymorphonuclear (gray arrow).

B. Ziehl Neelsen stain on histological sections

All slides are negative for Ziehl Neelsen stain.

Colonic resection

Macroscopic study to colonic resection piece (colectomy) showed a colonic rigid segment of 12cm in length, opening large areas of ulceration in cobblestones (gridling by cracks of a swollen mucosa). The wall was thick with a firm consistency (stenosing), in addition to more or less deep ulcerations, the mesos were the seat of sclero-lipomatosis and the lymph nodes were enlarged. The mesentery was usually thick, retracted and fibrous (Fig. **2**).

The resection specimen showed on microscopic study, a colonic mucosa with large trans-parietal ulcerations accompanied by a dense and diffuse, acute pyogenic and subacute polymorphic inflammatory granuloma rich in plasma cells and lymphocytes. Noting pronounced vascular congestion, streaks and lymphoid follicles in the mucosa.

Absence of epitheloid follicles within the boundaries of the room; the serosa is the site of fibro-lipomatosis. This lesional aspect was focal, it contained areas of differentiated and edematous local mucosa (Fig. **3**).

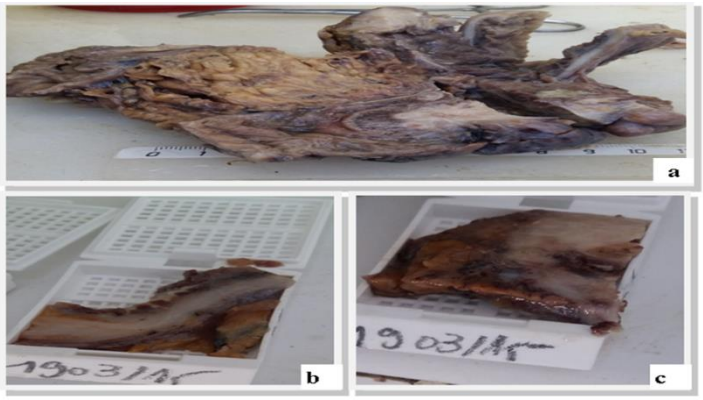


Figure 2. Macroscopy of the colonic resection piece and the selected sections.

a: Photograph of a 12cm rigid surgical specimen representing sclero-lipomatosis on its macroscopic appearance.
b and **c:** Macroscopic sections of the colonic resection specimen obtained by dissection of the stenotic parts.

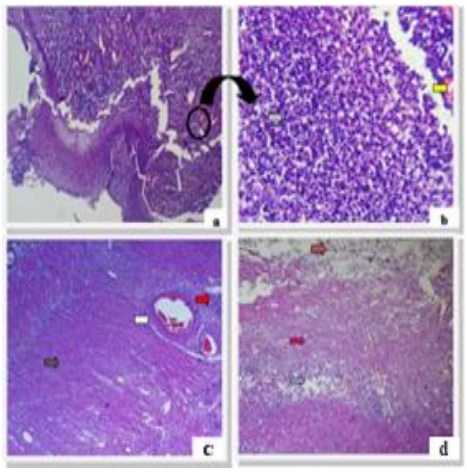


Figure 3. Histological sections of the colonic resection piece.

a: HE stained section seen at low magnification (40). The extensive inflammatory granuloma represents large ulceration rich in polymorphonuclear.

b: The section shows a zone rich in polymorphonuclear cells accompanied by pronounced vascular congestion (× 40) (yellow arrow).

c: HE stained section seen at low magnification (40), depicts perivascular fibrosis (white arrow), inflammatory granuloma (light red arrow) and slightly inflamed muscle layer (black arrow).

d: HE-stained section seen at low magnification (40), shows a layer of fat on the serosa (dark red arrow) and an aspect of fibro-lipomatosis (light red arrow) with the presence of lipid droplets.

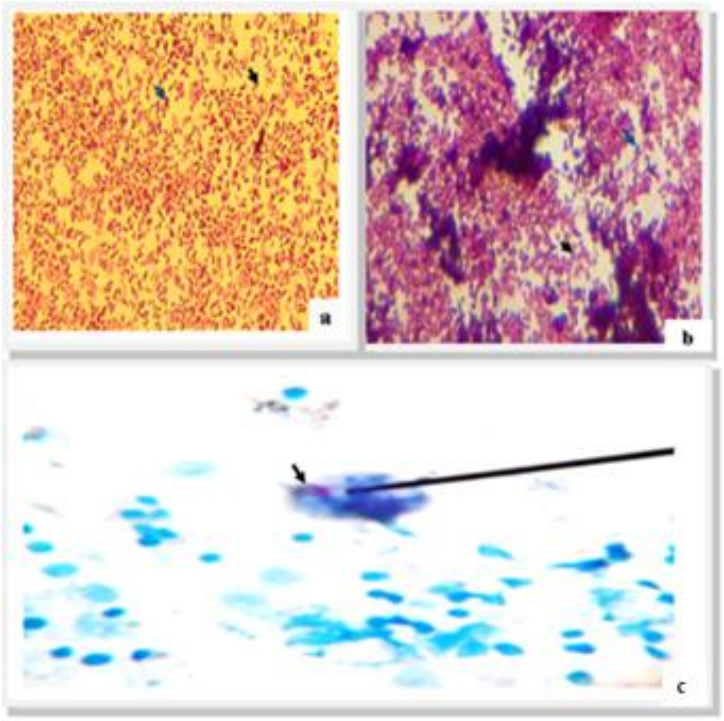


Figure 4. Bacteriological examination before culture.

a and **b:** Smear of the biopsy suspension before culture stained by the Gram method, seen under an optical microscope at 1000, shows a bacterial diversity of Gram-negative bacilli (black arrow) and Gram-positive (blue arrow) as well as Gram cocci positive (red arrow).

c: Biopsy smear stained by hot Ziehl Neelsen method seen under light microscope at 1000. With different lighting, Map is present inside macrophages appear as small curved bacillus stained red (black arrow).

Bacteriological examination of intestinal biopsies

In the fresh state, scattered epithelial cells were observed with numerous red blood cells, leukocytes and macrophages. In some cases, germs (cocci and motile bacilli) were observed. The Gram was verified, of which we observed Gram+ and Gram- bacilli, and Gram+ cocci arranged in a chain. Among the 15 biopsies taken, 2 cases were found to be positive for Ziehl stain, AFB present in the smear as small curved bacilli stained red. They were few in number (Fig. **4**).

In all the cases studied, after 24 hours of incubation at 37°C, pure colonies of similar salmon color were observed on Hektoen agar over the entire surface of the agar.

Blue-green colonies with a black center were isolated on Hektoen agar, Gram-bacilli had been observed, these bacilli could be *Salmonella* spp. or *Proteus* spp.

In Macconkey medium, lactose fermentation changed medium color to red. The strains were Lac+ where the colonies were mucous in appearance, gelatinous in consistency, larger in size, with a tendency to confluence (*Klebsiella* or *E. coli*).

Among the biopsies taken, 5 out of 15 cases gave a positive result in BGB (Fig. **5**). On blood agar medium, small, translucent colonies with total haemolysis were observed. These colonies were Gram+ cocci arranged in a chain (streptococci) (Fig. **6**).

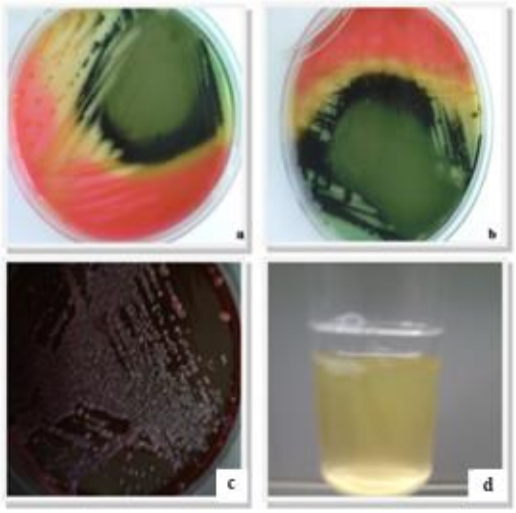


Figure 5. Results of the culture obtained after incubation at 37°C for 24h.

a and **b:** Culture of the biopsy suspension on Hektoen agar; Lac^+ strains (salmon) and H_2S^- strains (Green) were observed. Other H_2S^+ strains (black center).

c: Culture obtained from Macconkey medium.

d: Bacterial growth on BGB.

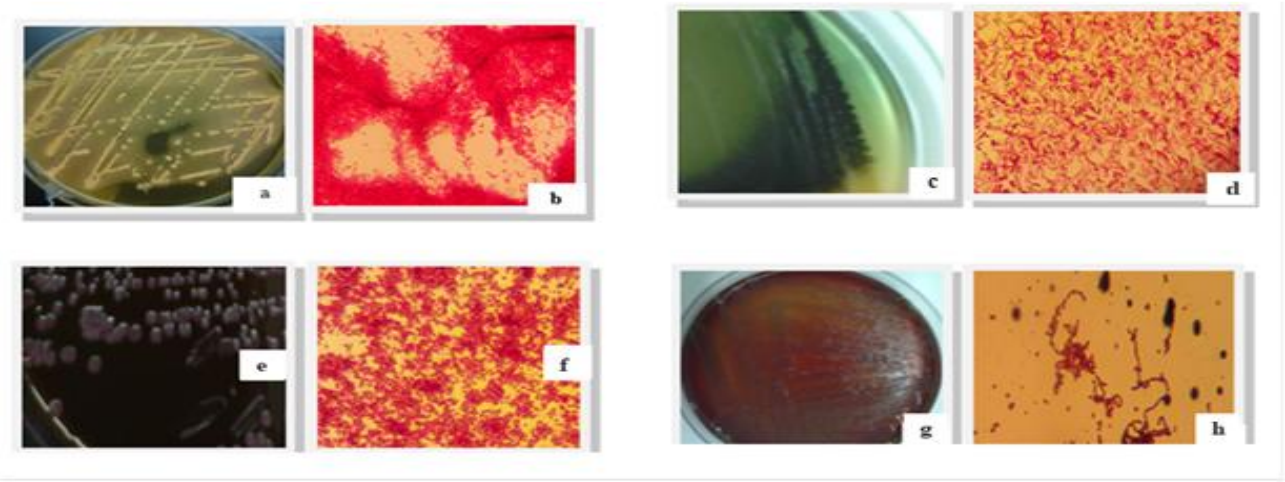


Figure 6. Macroscopic and microscopic identification of isolated bacteria

a: Isolation of *E. coli* on Hektoen medium, salmon colonies and mucous appearance.

b: Gram-stained smear observed under light microscope (100) represents Gram-negative coccobacilli.

c: Isolation of H_2S^+ colonies (Green with black center).

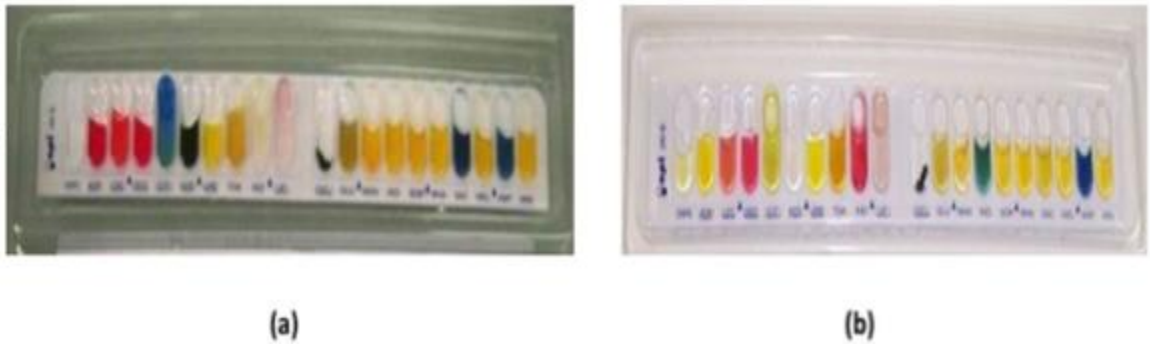
d: Smear represents Gram-negative bacilli after Gram staining, seen under an optical microscope (100).

e: Isolated colonies on MacConkey medium.

f: Smear represents Gram-negative bacilli after Gram staining seen under an optical microscope (100).

g: Colony isolated on blood agar medium.

h: Smear showed Gram positive chain sections after Gram staining, viewed under light microscope (100).



(a) (b)

Figure 7. Results of identification by the Api20E Gallery.

a: Api20 identification gallery after 24h/37°C incubation, followed by the code sheet which presented the biochemical characters of *Salmonella* spp,

b: Api20 identification gallery, reader after incubation at 37°C /24h showed the biochemical characteristics of *E. coli*.

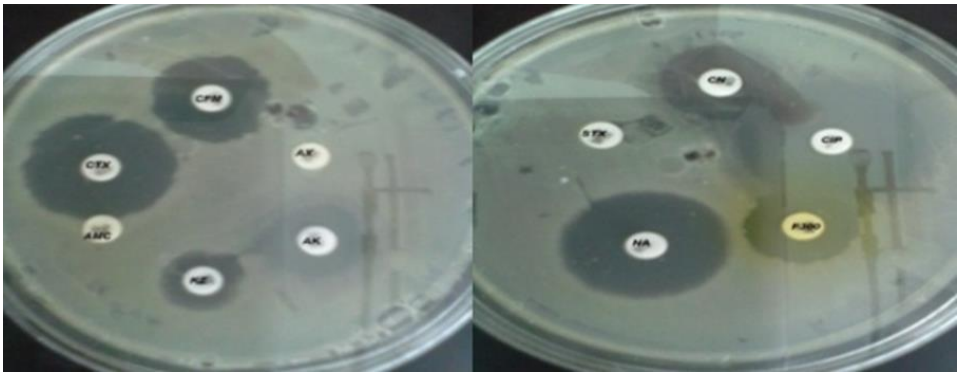


Figure 8. Antibiogram of *E. coli* after incubated at 37°C for 24 hours on MH agar.

a: Presents 6 discs of antibiotics of AMC; CTX; CFM; AX; AK and KZ

b: Presents 5 discs of antibiotics of STX; NC; CIP; F300; N / A.

The results obtained by the API20 galleries showed in Fig. **7**; after reading the results by the apiwebTM software; we had identified the two species.

Antibiogram

E. coli showed sensitivity for third generation cephalosporins (CTX; CFM), aminoglycosides (AK; CN) and also for quinolones (CIP; NA); and resistance for first generation cephalosporins (KZ), B-lactams (AMC; AX) signified the presence of B-lactamases, for sulfonamides (STX) and intermediate for Nitrofurans (F300) (Fig. **8**)

Coproculture

In stool analysis results were found similar to that of intestinal biopsies except that the Ziehl Neelsen stain was negative. We identified the same bacterial species determined by culture of intestinal biopsies, these species were: *Escherichia coli*, *Salmonella* spp., *Proteus spp. and Klebsiella spp.* The count of isolated bacteria showed a decrease in stool (*Salmonella* spp., *Proteus* spp. and *Klebsiella* spp.). On the other hand, *E. coli* was uncountable that indicated *E. coli* dominancy in CD.

DISCUSSION

Crohn's disease is Inflammatory Bowel Disease affecting the digestive tract. This IBD was examined in association with clinical symptoms followed by serological exploration which showed the signs of inflammation (high ESR and CRP). We also noted the radiological exploration which was very important, during the endoscopy the doctor took intestinal biopsies for the purpose of the anatomopathological analysis which helped in the orientation of the diagnosis.

In our study, we analyzed endoscopic biopsies to understand CD pathophysiology association with the intestinal microbiota, followed by histopathology of the disease and bacteriological analysis.

Crohn's disease is recognized by macroscopic bowel which reveals thickened and stiff mesentery with WAT in retrospective study of bowel resections[10]; mesenteric fat is defined as an extension of the WAT that covers a small and large intestinal circumference[11], the mesentery is generally retracted and fibrous and the intestine, with its surrounding, is thickened and enlarged with fatty lobules, and the mesenteric lymph nodes are inflamed[12]. All of these characters were presented in our study piece it is the appearance of sclero-lipomatosis, packing fat has been correlated in change of structures[11].

In the present work, we were able to compare between the results of the histological aspects of the intestinal biopsies and those of the resection piece, and by coming to the conclusion that the mucous biopsies showed only one tunic of intestine unlike the operating piece which represented the different coats of mucous membrane down to the serosa; therefore the mucosal biopsies obtained by endoscopy had not shown all the characteristics of CD that made possible to evaluate the disease (activity and identification of precancerous lesions), but not an exact diagnosis of the disease. The results must be associated with radiological, serological and clinical exploration [12,3]; because CD lesions is recognized "recognition of dysplasia". When surgical specimens are available, the disease can be much more easily identified [11].

Histological criteria are known in CD, including a diffuse transmucosal lymphoplasmacytic infiltrate associated with large and fissured ulcerations [3,12]. Cryptitis and crypt abscesses [13], lymphangiectasias [14] and perivascular inflammation [3] due to abnormalities in mWAT of CD patients which also included marked macrophages in the infiltrate and fibrosis also thickening of the vessels [11], visceral adipocytes are smaller in adipocytes through mesentery CD patients not in group control [15].

The hypothesis for the association of fat packaging and CD is that local production of mediators by mWAT could promote intestinal mucosa damage [11]; the relationship between mesenteric fat and CD needs to be investigated. Although CD is believe has an infectious cause autoimmune in origin[16]. Many works have been conducted to identifying the microbial diversity of healthy individuals and CD patients, in which a decline in bacterial diversity is found[17].

CD occurs in subjects genetically predisposed to inappropriate intestinal mucosal immune system activation and its stimulus is the intestinal bacterial content "microbiota"[18]. The intestinal microbiota contains approximately 10^{14} bacteria of various species, Gram staining used on fresh biopsy smears showed a great bacterial diversity. A growing interest centered on the study of bacterial communities as an antigenic source fueling chronic inflammation in CD [18].

The intestinal microbiota was identified by culture. 70% of this microbiota was not cultivable, it would remain unexplored [19]. The study of several bacteria associated with intestinal biopsies taken from CD patients shows that CD is a bacteriologically distinct disease [18]. The gut microbiota membrane favors sources of FA with which the immune system is dysfunctional [20].

Fecal microbial populations differ significantly from mucosal associated microbiota (MAM), and each individual harbors a unique flora pattern [21,22]. The analysis of the faecal microbiota remains informative, most often colonic and ileal biopsies are necessary for identifying MAM [18].

Biopsies used from healthy and pathological mucosa did not show any differences. In this work, a difference in bacterial composition was not due to the condition of the inflamed tissue, as bacterial collections associated with biopsies did not differ in composition. According to Vasquez et al. [23], there was no difference in injured and healthy areas of MAM.

The gut microbiota could carry pathogenicity in two ways: through pro-inflammatory or through anti-inflammatory; two complementary strategies were followed: the search for a candidate pathogenic microorganism and the analysis of the intestinal microbiota "search for dysbiosis" [18].

Many researchers have demonstrated that bacteria concentration in mucosa is higher in CD patients, in distal ileum and colon, precisely the concentrations of enterobacteriaceae [24,25]. One study showed that when the disease is active, the number of coliforms increased in faeces of CD patients and lowered in subjects recovering[26], and an increase in faecal enterobacteriaceae in the MAM

[24,25] such as *E. coli*[25]. Identification of ileal MAM of CD patients has indicated that *E. coli was* widespread, representing approximately half of total aerobic anaerobic bacteria[27], *Escherichia coli* charge has increased in MAMin patients compared to symptomatic subjects controls [28]. The strains were able to colonize the intestinal mucosa by adhesion to intestinal epithelial cells[26]. Immunocytochemistry demonstrated that *Escherichia coli* AG was found in resections of most intestinal CD patients[26].

High levels of anti-OMP *Escherichia coli* of CD patients appeared in bowel surgery such as stenosing and perforating[18]. Certain *E. coli* strains containing particular adhesion have been found in ileal mucosal samples from CD patients [27]. This strain of *E. coli* isolate did not contain the virulence factors of other AIEC pathogenic *E. coli* (reference strain LF82), they are generally strongly infected immune cells secreting high levels of TNF-α [26]. AIEC (LF82) adhere to enterocytes of CD patients [29] and colonize the ileal mucosa, and phagocytosed and multiply in lamina propria, induce strong secretion pro-inflammatory cytokines TNF-α [18]. The greater AIEC richness was due to the stability of bacteria in CD patients mucosa [30,31,32].

AIEC was capable of inducing the formation of granuloma *in vitro*[26,29]. Researchers suggested that optional pathogens cause disease in susceptible individuals [33]. In addition to the high concentration, we noted the presence of unusual bacteria [34,35] such as those found in our study, including *Salmonella*, *Shigella*, *Streptococcus*, *Proteusmirabilis*, *Klebsiella*, *Mycobacterium avium paratuberculosis*. Autophagy which is a process of degradation the damaged organelles by lysosomal eukaryotic [36]. It limited growth intracellular bacteria [37,38]. It's dysfunction conducted to infection like *S.typhimirium*, *S. pyogenes* also *Mycobacteria*[39,38,40], lesions seen in this disease are mostly present at the Peyer's patches. For which many bacteria have a particular tropism such as *Salmonella*, *Shigella*, *Vibrio*, *Campylobacter*, *Yersina*, *Lesteria*, *Klebsiella*[41]. Aphthous ulcerations resulting from M cell necrosis, which are lesions in CD patients. These ulcerations occur in many infections by *Shigella*, *Salmonella* also *Yersina*, invasion is an element of virulence[26].

Most possible infectious cause is *Mycobacterium avium paratuberculosis*[16], it may be there is association between Johne's and Crohn's diseases[42]. MAP is increased in adults Crohn's disease[43] and newly diagnosed children [44], the mycobacterial theory and the self- immune system in CD are complementary, the first concerns the etiology of the disease and the second concerns its pathogenesis[5].

MAP has been studied in tissue of many CD patients [45]. Unlike other environmental mycobacteria, MAP can causing chronic inflammation of the intestine [45].

MAPs are difficult to diagnose by Ziehl- Neelsen staining, sometimes culture or DNA or RNA detection of MAPs is doing in laboratory[16]. Mucosal biopsies were obtained from CD patients by endoscopy used for smear and examined by ZN. Smears of all patients were negative by ZN, after 14 weeks cultures MGIT shown in ZN of the characteristic cells and AFB [43], if the shape of cell wall is deficient in MAP, the ZN will be negative. In prolonged period of culture (weeks to years) MAP can produce a protoplasmic [46,16], and ZN staining will be positive [16] . Important role, moreover the lifestyle and the diet of the people affected little appear modifications in the cell wall of the MAP. While geographical and environmental conditions can give resistance to this strain, it is therefore proposed that strains of MAP can differentiate according to environmental conditions. The use of ZN staining on histological sections gave negative results, while their use on biopsy smears in the same patients appeared positive in two cases. It is concluded that this method is more effective on smears than on histological sections. In animals, tissues stained with ZN for the direct visualization of mycobacteria in the study by Wells *et al*. [47] who identified AFB in 9% and 5.6% of cases respectively in the ileal mucosa and the lymph nodes. It is a method that requires a lot of specific manipulations and expertise in anatomo-pathology. For the study by Weber *et al*.[48] which was based on the ZN technique on a matrix smear on a "smear" glass slide, a sensitivity of 48% and a specificity of 98% for animals in the clinical phase was established.

NOD2 an intracellular sensor of bacterial peptidoglycan [18], in mutations NOD2/CARD15 gene of CD patientsinnate immune response a disease by invasive bacteria [49], NOD2 intervenes in clearance of *Salmonella* spp., streptococci [50,51] also *Enterococcus*[52]. Monocytes and cells epithelialexpress NOD2[53], intracellular receptor for muramyl dipeptide[53]. NOD2 is also expressed in cells paneth [54] synthesized and secreted several antimicrobial

peptides, including lysosomes, phospholipase and defensin. These peptides are sensitive to oral administration of pathogenic bacteria, likewise provide protection against *Salmonella*[55]. Studies showed biological effects of the peptides on pathogenic lumen microbiota gut [56], defensin is essential element CD pathophysiology[57]. Defensins, in deficiency in innate immune defense, inducing adherence of bacteria in mucosa conducted to inflammatory response in CD[58].

CONCLUSION

In our study, the common histopathological appearance of CD is defined by the presence of inflammatory granuloma with clear-centered lymphoid follicles (rich in lymphoplasmocytes). The use of Ziehl Neelsen staining, for detection of MAP, is effective on smears as well as on histological sections, it must be followed by culture with meta-analysis and PCR for consistent results. The results obtained on the intestinal microbiota in patients with CD are different, on one side of increased concentrations of certain bacteria such as *E. coli* and *Proteus mirabilis*; and on the other hand the appearance of certain unusual pathogenic bacteria such as *Salmonella*, *Shigella*, *Klebsiella* and *Streptococcus pyogenes*. The results are due to the deficit in immune regulation caused by the NOD2/CARD15 gene mutation.

Over the last few years, IBD has become very frequent in Algeria. Our perspective is to provide research groups for the study of the pathophysiology and especially the etiology of the disease which is different according to the geographical areas. We also want to conduct more research on MAPs which we assume are more resistant and different from the reference strains and to introduce new animal and human vaccines against MAP.

ETHICAL APPROVAL

Ethical approval for the study was obtained from Khemis Miliana University, Algeria.

CONFLICTS OF INTEREST

No conflict of interest.

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LIST OF ABBREVIATIONS

CD: Crohn's disease

BGB: Buffered glucose broth.

MAP: *Mycobacterium avium paratuberculosis*

IBD: Inflammatory Bowel Disease

NOD2: Nucleotide-binding Oligomerization Domain 2.

CARD/15: Caspase Recruitment Domain-Containing Protein 15.

PCR: Polymerase chain reaction

ZN: Ziehl Neelsen

HE: Hematoxylin Eosin

BHIB: Brain Heart Infusion Broth.

DNA: Deoxyribonucleic acid

MGIT: Mycobacteria Growth Indicator Tube

RNA: Ribonucleic acid

AFB: Acid Fast Bacillus

Lac: Lactose

AIEC: Adherent Invasive *Escherichia coli*

TNF-α: Tumor Necrosis Factors-alpha.

OMP: Outer Membrane Protein

AG: Antigen

MAM: Mucosal Associated Microbiota

WAT: White Adipose Tissue

API20 E: Application Programming Interface 20Test Enterobacteriaceae.

UHC: University Hospitalier Center

CRP: C-Reactive Protein.

E. coli: *Escherichia coli*.

ESR: Erythrocyte Sedimentation Rate.

S.typhimirium: *Salmonella typhimirium*

S.pyogenes: *Streptococcus pyogenes*

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