INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print) 2222-5234 (Online)
http://www.innspub.net
Vol. 14, No. 2, p. 521-535, 2019

RESEARCH PAPER OPEN ACCESS

Contribution to the evaluation of gastric microbiota, case of B gastritis

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Key words: Gastritis, Biopsies, Culture, Bacteria, Biochemical tests, Phenotypic identification

http://dx.doi.org/10.12692/ijb/14.2.521-535 Article published on February 28, 2019

Abstract

The present study focused on the isolation and identification of the gastric bacterial ecosystem involved in the pathology of B type gastritis according to Sydney classification. Isolation performed after grinding the various biopsies on specific selective culture media permitted a selection a set of bacteria belonging to this gastric ecosystem. The data were phenotypically identified, by biochemical assays use, different API galleries. The main isolated germs belong to the genus and species, Staphylococcus aureus, Proteus-mirabilis, and Helicobater-pylori.

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Introduction

The gastrointestinal tract is an ecosystem, favorable to exogenous microorganisms. It is managed by a cooperation between the gastrointestinal epithelium, the immune system, and the microbiota commonly called intestinal flora. Interactions between microorganisms and the host can be of three types: symbiosis, commensalism and pathogenicity (Hooper and Gordon, 2001).

The microbiota of the gastrointestinal tract has been estimated at about 1013-1014 microbial cells representing 400-500 species and subspecies (Moore and Holdeman, 1974; Björkstén, 2004).

Bacteria have specific respiratory needs, they are either strict aerobes requiring oxygen for their breathing, or strict anaerobes whose presence of oxygen is toxic to them, or facultative aero-anaerobes that can develop in the presence or absence of oxygen. In the absence of oxygen, either micro-aerophiles that develop under a low oxygen pressure, or finally aero-tolerant anaerobes developing in the absence of oxygen, but they tolerate the presence of the latter in the medium. (Rolfe *et al.*, 1978)

The gastric microenvironment is home to acidotolerant and facultative anaerobic microorganisms such as lactobacilli, streptococci, and yeasts (Cummings *et al.*, 1989; Gournier-Chateau *et al.*, 1994)

The gastric ecosystem hosts a pathogenic bacterium called Helicobacter pylori which is recently recognized for its inducing power of certain gastric pathologies, such as gastritis, gastric ulcer, gastric lymphoma MALT and also gastric cancer (Mégraud, 1994).

The objective of this study is to isolate, identify the different microbiota, and look for possible anaerobic bacteria coexisting with Helicobacter pylori, and also aerobic tolerant bacteria, and this from the gastric biopsies of patients, taken at of the gastroenterology department of Mostaganem Hospital, and private offices.

After endoscopic examination, one of the biopsy samples is intended to undergo an examination of pathology to confirm the endoscopic diagnosis of gastritis or gastric ulcer, the other samples will be intended for the microbiological study.

After the isolation step, biochemical tests for bacterial identification and the use of the different API galleries indicated for each of the isolated bacterial strains were performed.

Materials and methods

Endoscopic examination

Biopsy specimens were taken away from treatment with antibiotics or antisecretory agents. The conditions of sterility must be respected at the time of sampling. The samples were taken under endoscopy in the antral region about 2 cm from the pylorus using a standard endoscope (Olympus GIFXQ 10) and a biopsy forceps, previously disinfected with glutaraldehyde and rinsed well due to inhibitory activity of this product on Helicobacter pylori (Miftahussurur and Yamaoka, 2016)

(Mégraud and Lamouliatte, 1992) Four (04) antral biopsies were taken, which will be respectively used for cytology, histopathology and culture. The biopsy specimens are placed in sterile dry tubes containing saline or saline saline.

Pathological examination

The purpose of this examination is to confirm the endoscopic diagnosis. It allows on the one hand, to study the gastric mucosa and its histological modifications, and on the other hand to visualize the spiral bacteria. It was performed on histological sections prepared from gastric biopsies, stained with modified Giemsa stain, and observed at high magnification (Mégraud, 1995)

Microbiological examinations

Cytological examination

This examination consists of the demonstration under anaerobic and aerobic conditions of the germs of different forms, belonging to the gastric ecological

niche, and which can testify to the presence of bacterium, by a Gram stain of the biopsic imprint. For this, a biopsy is deposited on a slide, then crushed with a second blade in order to obtain a fine and regular smear. The prepared smear is then dried, fixed, and then stained with Gram stain (Sobhani *et al.*, 1991; Mégraud, 1994; Cellini *et al.*, 1995). Then, the preparation is observed under a light microscope at low magnification (X 10) in order to visualize and locate the richer areas in cells, then at high magnification to observe the different Gram stains, and selectively the negative Gram spiral shapes. It is necessary to observe a large number of microscopic fields because of the sometimes inhomogeneous distribution of the bacteria in the gastric biopsy (Megraud, 1989; Mégraud and Lamouliatte, 1992)

Culture and isolation

For isolation, gastric antral biopsies are crushed in 1 ml of nutrient broth using a sterile mortar to release the bacteria and then seeded in modified Columbia agar medium with 10% blood mutton, and this after successive decimal dilutions. The fresh state consists of depositing on a slide, a drop of the bacterial suspension covered by a coverslip in search of possible mobility of the microorganisms, the preparation is observed at magnification (x 40). (Marchal *et al.*, 1991). After inoculation, few boxes are immediately incubated in a jar in a microaerobic atmosphere to promote the development in anaerobiosis, and the other boxes are put directly into the incubator to allow growth in aerobic at 37 ° C for 5-7 days. The atmosphere is renewed at least every two days for optimal growth.

Identifications

Identification is based on the determination of morphological and biochemical characters (Cassel, 1996).

Macroscopic examination

The morphology of the colonies and their size are studied from the cultures obtained on the following media: Colombia agar or chocolate agar (Mégraud and Lamouliatte, 1992), (Fauchère and Rosenau, 1991).

Microscopic examination

It was performed on a bacterial smear, prepared from suspicious colonies in pure cultures, then fixed and stained by the Gram method. (Mégraud, 1994).

Biochemical tests

Catalase test

Catalase is an enzyme produced in abundance by bacteria with a respiratory metabolism that destroys hydrogen peroxide and releases oxygen, (Vezina *et al.*, 1991; Lacroix *et al.*, 2000)

The technique involves taking a portion of the colony and emulsifying in a drop of hydrogen peroxide (30 V). The release of gas bubbles means that it has catalase production (Prescott *et al.*, 2003).

Oxidase test

The oxidase assay is based on the bacterial production of an intracellular oxidase enzyme in the presence of atmospheric oxygen and cytochrome C (Vezina *et al.*, 1991; Lacroix *et al.*, 2000).

To determine the oxidase activity, a taken colony is put in a drop of oxidase reagent (Biomerieux France). The development of a purple color means that the test is positive and that the isolate has the oxidase enzyme (Kovács *et al.*, 1995).

Urea rapid test

A biopsy fragment is put using a sterile loop in a tube containing 0.5 ml of urea-indole solution. The reading of the results is interpreted by a turn of the indicator towards red, taking place after 20 min and 24 h after incubation at 37° C. (Cassel-Béraud *et al.*, 1997).

Triple Sugar Iron medium (TSI)

This method makes it possible to demonstrate on the one hand, the fermentation of glucose (with or without release of gas), lactose, sucrose and on the other hand, the production of hydrogen sulphate (H2S). It is a sloping and pellet medium by seeding a bacterial colony, the slope is seeded by streaks and the pellet by central bite, then incubation at 37C° for

18 to 24 hours, and this for the purpose of evaluating the metabolic activity of different bacterial strains, such as, the lactose fermentation on the slope that translates to yellow turn, the fermentation of sucrose which is also visualized by a yellow turn, the presence of gas that is manifested by the detachment of the pellet or the presence of air bubbles, and the production of H2S which is interpreted by a black coloring. (Marchal *et al.*, 1991; Leveau and Bouix, 1999)

Mannitol-mobility test. (Le Minor, 1993)

Inoculation of the environment is realized by central sting with the Pasteur pipette buttoned and loaded with a pure ba sting cterial suspension, then put into oven at 37° C for 24 hours.

The use of mannitol acidifies the medium which can be revealed by turning the pH indicator to its acidic (yellow) hue.

If the bacterium ferments mannitol, the reaction is called positive(+) mannitol, on the other hand, if the medium turns to red, no fermentation of mannitol, the reaction is called: negative (-) mannitol.

Fermentation of mannitol with gas production

The presence of bubbles indicates a presence of gas, it is said that the reaction is called positive mannitol (+) with gas production.

Mobility

The immobile germs only grow at the level of the central sting while the mobile germs diffuse in the agar.

Search for nitrate reductase

Two (02) drops of Nit1 and Nit2 are added in the different tubes, if there is a red ring it is said that we have a positive nitrate reductase (+) reaction, but if there is no reaction, we will proceed to the addition of Zinc powder:

If there is no red ring, no reaction, it is the stage of dinitrogen, one says nitrate reductase positive (+). But if there is presence of red ring, it is called nitrate reductase negative (-).

Interest of API galleries

The study of biochemical characters is based on the use of API galleries. 20E, 20NE, which will be used to perform specific biochemical tests for each isolated bacterium (Fabre *et al.*, 1994)

Antibiogramme

This test makes it possible to study the sensitivity of isolated strains to antibiotics, this sensitivity is tested by the dissemination method (disk method) on Muller-Hinton medium (MH) supplemented with 10% of sheep blood, using several antibiotics (Lozniewski *et al.*, 1996)

Bacterial Interactions

In this section we will test the inhibitory effect of *Lactobacillus sp.*, Selected against Gram-positive and Gram-negative bacteria found in our research study. The lactic strains are tested for their inhibitory power with respect to certain pathogenic bacteria, thus making it possible to determine their spectrum of activity (Geis *et al.*, 1983).

Results and discussion

Endoscopy examination: (Fig, 1 and 2).

The seat of inflammation is characteristic for each patient, it is either antral or fundic. The endoscopic diagnosis is that of erythematous gastritis with a congestive fundus and antrum, sometimes with bilious reflux.

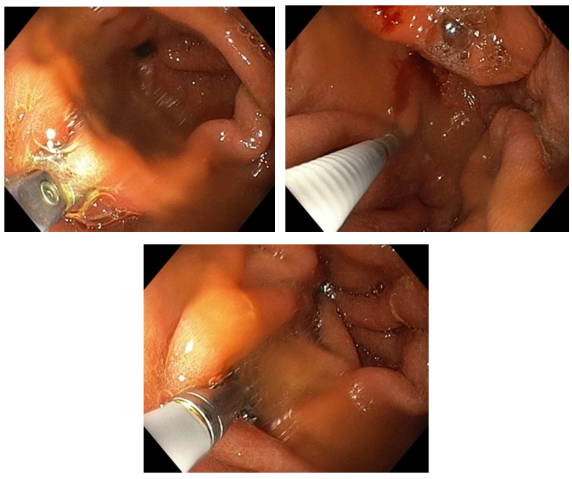


Fig. 1. Collection of a biopsy of an erythematous antrite.

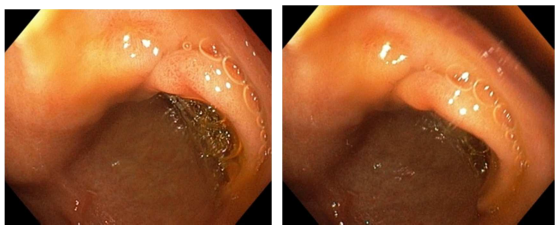


Fig. 2. Exulcerous Antrite.

Pathological examination

Histopathological examination of microfragments revealed chronic atrophic gastritis, with occasional observation of *Helicobacter pylori* in the form of comma (,), V, and curved (Fig. 3). On the other hand, it has been noted in the Fig, 4 only lesions of the gastric mucosa in favor of gastritis, without the presence of the *Helicobacter pylori* forms.

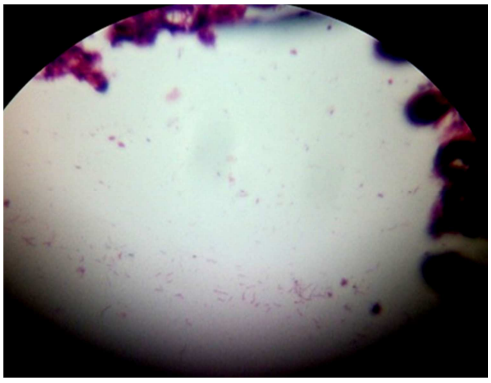


Fig. 3. Pathological microscopic observation of biopsy fragment with presence of Helicobacter pillori.

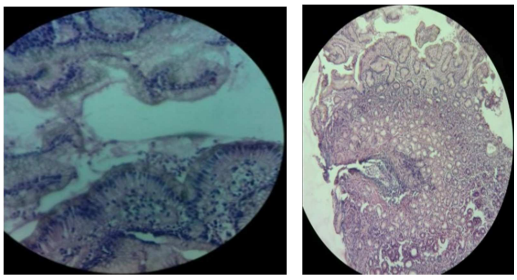
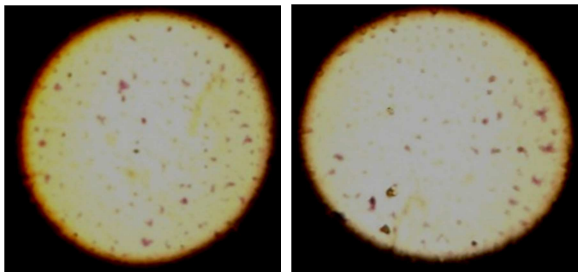


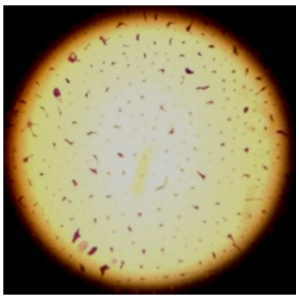
Fig. 4. Pathological microscopic observation of gastric biopsy fragment (Gr X 100).

Cytological examination of the biopsy

After Gram staining of the gastric biopsy impression, the observation under the optical microscope at x100 magnification reveals the presence of Gram-negative bacillus in the form of V (Fig, 5a), Virgule (Fig, 5b), curved (Fig, 5c), and cocoid (Fig 5 a, b, c).



a. Shape: V b. Comma form (,)



c. Shape: Curved

Fig. 5. (a, b, c). Optical microscope observation of a human gastric biopsy fragment, X100 magnification.

Culture and isolation

The observation in the fresh state, under the optical microscope at low magnification x 40, reveals that some bacteria are mobile, while others are immobile.

Macroscopic examination

After seeding from the different decimal dilutions from the crushing of human gastric biopsies, on different selective isolation media, and after aerobic and anaerobic culture in the oven at 37° C, there is an appearance of bacterial colonies of different diameters, the smallest of which can reach a millimeter (1 mm), of rounded and sometimes convex shape, light brown and dark, creamy, transparent and shiny. These macroscopic characters are characteristic of different colonies of aerobic and anaerobic bacteria (Fig. 6).

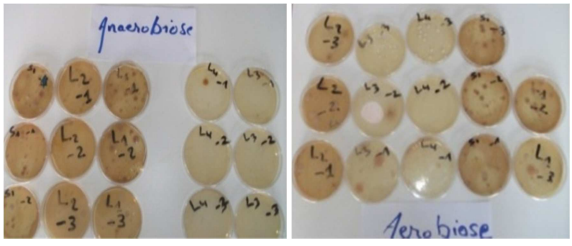


Fig. 6. Anaerobic and aerobic culture of bacteria on solid media colombia with blood.

Microscopic examination

In order to obtain pure bacteria, it was necessary to make several transplants, and following a gram stain, the observation under the light microscope at high magnification (x 100), revealed the presence of several microbiological Gram forms. Positive (+) and Gram negative (-) from aerobic culture (Fig 07), and anaerobic (Fig 08) that can be either bacilli or coccis.

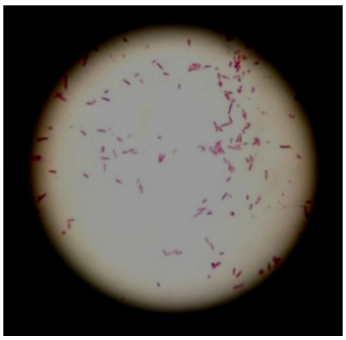
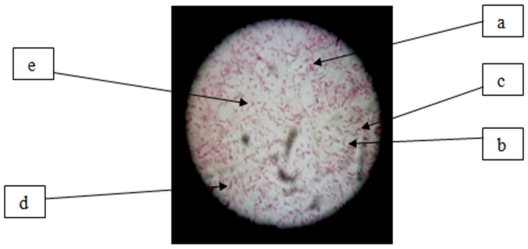
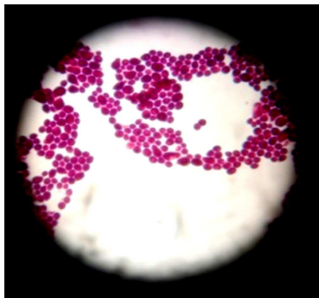


Fig. 7. Microscopic appearance of different aerobic isolate (S1, 2). (Isolate S1, 2 small bacilli and Gram negative cocobacillus).



Isolate L2, 1 Gram-negative Bacillus in different aspects, in V (a), comma (b), S (c), slightly curved (d), and coccoid (e).



Isolate L3 Gram (+) cocci (+), associated, grouped together into clusters.

Fig. 8. Microscopic appearance of different anaerobic isolates (L2, 1 , L3).

Results of the antibiogram test

Aerobic isolates

Table, 01 interprets the results of the antibiogram of aerobic bacteria isolates, S1, 2. The study of the sensitivity of the different bacteria tested with the antibiotics usually used in the therapeutic choice by the disk diffusion technique showed the excellent activity of the majority of the antibiotics used with respect to these bacteria (Fig, 09).

The bacterium S1, 2, is sensitive to, cefoxitin (FOX) (12 mm), amoxicillin + clavulanic acid (AMC) (18mm), gentamycin (CN) (26mm), and Kanamycin (K) (20mm), but presents a resistance to Cefotaxime (CTX) (08mm), Ampicillin (AM) (09mm), and Nalidixic Acid (NA) (07mm).

Table 1. Result of the antibiogram against aerobic bacteria.

Aerobic bacteria \ Discs of antibiotic	CTX (mm)	FOX (mm)	AM (mm)	AMC (mm)	CN (mm)	K (mm)	NA (mm)
S1, 2	08	12	09	18	26	20	07

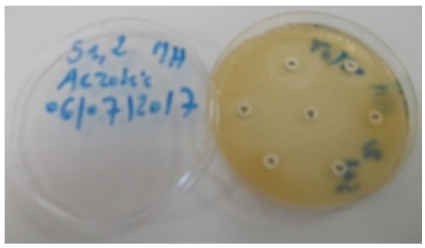


Fig. 9. Antibiogram of aerobic bacteria on Mueller Hinton medium (S1, 2).

Anaerobic isolates

Table, 02 interprets the results of the antibiogram of isolates of anaerobic bacteria, L2,1, and L3 (L3,1), (Fig, 10).

The bacterium L2.1 is sensitive to discs, Cefotaxime (CTX) (26mm), Cefoxitin (FOX) (12mm), Gentamycin (CN) (27mm), Kanamycin (K) (25mm), and Nalidixic Acid (NA) (22mm). It is resistant to Ampicillin (AM) and Amoxicillin + Clavulanic acid (AMC), respectively having a zone diameter of inhibition at 06 and 07 mm.

The L3 (L3,1) bacterium is resistant to the majority of discs with an inhibition zone diameter of 06 and 07 mm, but is slightly sensitive to cefoxitin (FOX) (13mm) and nalidixic acid (NA) (12mm).

Table 2. Result of antibiogram against anaerobic bacteria.

Discs of antibiotic / anaerobic bacteria	CTX (mm)	FOX (mm)	AM (mm)	AMC (mm)	CN (mm)	K (mm)	NA (mm)
L2,1	26	12	07	07	27	25	22
L3=L3,1	07	13	07	07	07	06	12

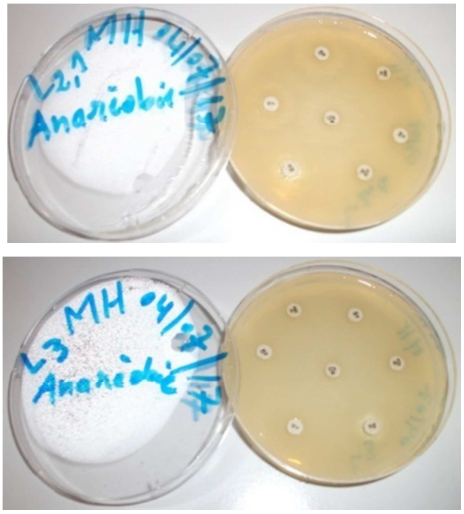


Fig. 10. Antibiogram of anaerobic bacteria on Mueller Hinton medium (L2, 1, L3).

Inhibitory effects of lactic acid bacteria

The interest of this test is the demonstration of possible antagonism existing between lactic acid bacteria (lactobacilli: LCA, B116, LV21 LV22) with respect to bacterial, aerobic and anaerobic isolates coexisting in the human gastric ecosystem. Table, 03, interprets the results of the antagonistic effect of lactic acid bacteria on the aerobic bacteria found in our research study (Fig, 11).

Table 3. Result of the inhibitory effect on aerobic bacteria.

lactobacilli / Aerobic bacteria	B116(1) Mm	LV21(2) mm	LV22(3) Mm	LAA(4) Mm
S1, 2	06	07	11	13

The bacterium S1, 2 is sensitive to LV22 (11 mm), LAA (13 mm), however it is resistant to B116 (06m) and LV21 (07mm).

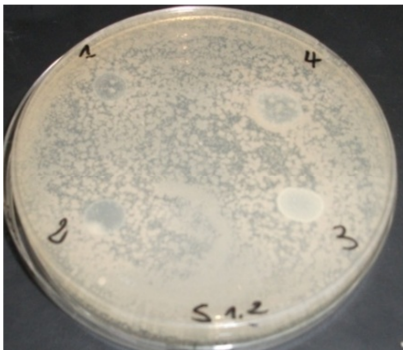


Fig. 11. Inhibitory effect of lactobacilli on aerobic gastric bacteria (S1, 2).

Table, 04, interprets the results of the antagonistic effect of lactic acid bacteria on anaerobic bacteria. (Fig, 12).

Table 4. Result of the inhibitory effect on anaerobic bacteria.

lactobacilli / Anaerobic bacteria	B116(1) Mm	LV21(2) Mm	LV22(3) Mm	LAA(4) Mm
L3= L3, 1	09	08	10	17
L2, 1	09	10	11	13

The bacterium L2, 1 is slightly sensitive to B116 (09mm), sensitive to LV21 (10mm), to LV22 (11mm) and LAA (13mm).

The bacterium L3 is slightly sensitive to B116 (09mm), resistant to LV21 (08mm), but sensitive to LV22 (10mm) and LAA (17mm).

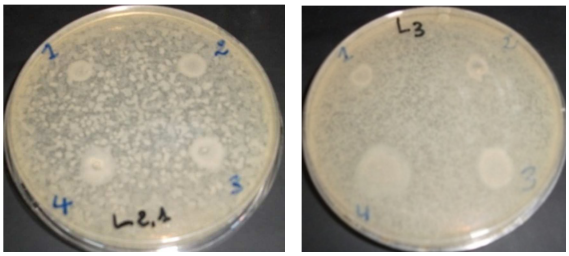


Fig. 12. Inhibitory effect of lactobacilli on gastric anaerobic bacteria (L2, 1, L3).

Criteria for identification

The identification of the isolates was performed against certain criteria obtained from the results, which are respectively related to the macroscopic appearance of the colony of the bacterium, the gram stain, the biochemical characteristics and the resistance phenotype. Antibiotics, the other biochemical characters being generally studied from API 20 NE, and API 20 E.

Anaerobic bacteria

The isolate L2, 1

The set of results that were found in the table, 05, directs us towards a pre-identification of the L2, 1 isolate as *Helicobacter pylori*. In parallel with the results of the table, 06 (biochemical tests), it was found that the table, 07 groups the phenotypic characteristics of the isolate L2, 1, in favor of *Helicobacter pylori*, referring, respectively, to the anatomopathological examination, cytological biopsy fragment in the fresh state, and microscopic study of the bacterium.

The results of the isolate L3 (L3, 1), grouped in Table 07, point to the genus *Staphylococcus*. In parallel with this phenotypic aspect, the yellow coloration in Chapman medium, and the positivity of the coagulase test adopt the strain *Staphylococcus aureus* for this isolate.

Table 5. Result summarizing the identification criteria for isolate L2, 1.

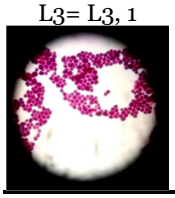
Critères / Anaerobic isolate	Gram	mobility	catalase	oxydase	Uréa	indole	mannitol	Respiratory type	Others
L2, 1	Gram negative	mobile	+	+	+	+	+	microaerobic	Lactose(-) sucrose (-) H2S(+) Gas (–) Nitrate(+)

Table 6. Result of the identification of Helicobacter pylori.

Criteria / Isolate	Optical microscope observation (x 100) ASPECT			mobility	catalase	oxydase	Uréa	Respiratory type
	Pathology	Fresh state	Microscopic					
L2, 1	V Y	V Incurved comma(,)	V S Incurved comma(,)	mobile	+	+	+	microaerobic
	Figure :3	Figure :5	Figure : 08					

The isolate L3 = L3, 1

Table 7. Result summarizing the identification criteria for isolate L3= L3, 1.

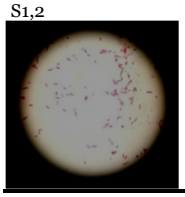
Tests / Anaerobic isolate	Gram	mobility	catalase	oxydase	Urea	indole	mannitol	Chapman culture	coagulase	Respiratory type	others
L3= L3, 1 	Cocci gram +	Immobile	+	-	+	+	+	+	+	Optional anaerobic air	Lactose + Gas -

Aerobic Bacteria

The isolate S1, 2

The set of results, grouped in the table 08, parallel to those obtained by the api20E gallery, made it possible to identify the isolate S1,2 as *Proteus mirabilis*: 7323000.

Table 08. Result summarizing the identification criteria for isolate S1, 2.

Tests / Aerobic isolate	Gram	mobility	catalase	oxydase	Uréa	indole	Mannitol	chapman Culture	coagulase	Respiratory type	Others
S1,2 	Cocobacillus Gram-	mobile	+	-	+	+	+	-	-	Optional anaerobic air	Lactose - sucrose - H2S - Gas – Nitrate -

Discussion

The realization of the gastric biopsies during fibroscopy followed by histological, cytological, culture, rapid urease test, and identification by other biochemical tests revealed a number of bacterial strains belonging to the gastric ecosystem.

In our research study we isolated several bacteria but the most considered are, Helicobacter pylori, Proteus mirabilis, and Staphylococcus aureus and are therefore predominant in the gastric microenvironment. Their presence at the gastric level is justified by their resistance to high gastric acidity by their secretion of a strong urease. Nevertheless, this is not the case of the strain Staphylococcus aureus which, despite having a negative urease, resists gastric acidity and this resistance is probably due to a variable and powerful genetic code.

These three bacterial strains secrete toxins capable of causing an inflammatory state in the gastric mucosa and thus induce their pathogenic powers that can generate an immune disorder to these patients.

The antibiogram has allowed us to make a choice towards other antibiotics that have the ability to render through their active ingredients these sensitive bacterial strains, which may lead to inhibition or weaken the effect of toxins secreted by these bacteria.

The use of the inhibition test by lactic acid bacteria derived from goat's milk led us to observe that the majority of these bacteria generally had a sensitivity towards the majority of these lactic acid bacteria, and could thus introduce it as a dietary supplement in combination with conventional treatment in the case of Gastritis B.

Bacteria ingested during meals have limited survival in the stomach (Vincent, Vachée, & Leclerc, 1995) with the exception of those that pass quickly through the stomach or that are resistant to gastric pH (Savage, 1977). Indeed, the cases of *Streptococcus entericus,* bacterial colonization of the stomach by species of aerodigestive origin (*Streptococcus, Lactobacillus, Staphylococcus, Bacteroides, Bifidobacteria* ...), and occasionally *enterobacteria, pyocyanic bacilli* and yeasts (*candida*), may be due to age-related hypochloridemia, antisecretory treatment or surgery (Vagotomy, gastrectomy); (Bishop and Anderson, 1960; Savage, 1977; Drasar, 1989; Vincent *et al.*, 1995).

The stomach, a strongly acidic medium (up to PH 2) where oxygen is present, hosts acid-tolerant and facultative anaerobic microorganisms such as *streptococci* and *lactobacilli*, and a microaerophilic *Helicobacter pylori* (Lamine *et al.*, 2004). But they remain small (101 to 103 cfu / ml) because of the acidity and the intestinal motor activity which limits a stable colonization of the gastric epithelium. In the small intestine, the microbiota is composed of facultative

anaerobic bacteria such as *streptococci, lactobacilli and enterobacteria* and strict anaerobes such as *bifidobacteria, bacteroids* and *clostridia*. The flora is still relatively poor because of the peristalsis and the abundance of secretions; the density increases from 103 to 108 cfu/ml from the duodenum to the ileum. In the colon, the microbial flora is more and more varied and abundant (1012 cfu/ml). It is dominated by anaerobic bacteria (*Bacteroides* 1011 per stool gram, *Bifidobacterium, Clostridium*) while facultative anaerobic bacteria (such as *lactobacilli)* disappear almost completely (O'Hara and Shanahan, 2006).

The human microbiota is distributed mainly in the oral cavity and along the digestive tract but it is in the distal part of the digestive tract that the bacteria are in greater numbers. They are arranged according to a growing oro-anal gradient with maximum microbial richness in the distal colon. There would be a total of 1014 bacteria in the digestive tract, which is 10 to 20 times the total number of cells in all tissues of the body (Suau *et al.*, 1999).

S1,2 isolate (aerobic)

The overall results, had identify S1.2 isolate as *Proteus mirabilis*: 7323000. This strain is the usual host of digestive tract of man and animals. The strain *Proteus mirabilis* exhibits the general characteristics of *Enterobacteria*, it is an optional aeroana bacterium, fermenting glucose with gas production, H2S positive, oxidase negative, catalase positive, has a nitrate reductase, (Archambaud *et al.*, 2015).

Bacteria belonging to the species *Proteus mirabilis*, have a morphology of gram-negative rod-shaped bacilli, generally very mobile, polymorphic, measuring from 0.4 to 0.8µm in diameter over 1.0µm to 80µm in length, very alternately flagellated between vegetative swimmers and hyper-flagellate teeming cells (Belas *et al.*, 1991). *Proteus mirabilis* has the ability to elongate and secrete a polysaccharide when in contact with solid surfaces, making it extremely mobile on items such as medical equipment. It is characterized by its motility of swarming, its ability to ferment maltose and its inability to ferment lactose (Liu, 2009).

Proteus mirabilis requires an environment with high alkalinity. A suitable medium for *Proteus mirabilis* must have a pH greater than (Frasca *et al.*, 2008). Nevertheless, it was found in our study, that this bacterium had presented a positive urease following the urea indole test, allowing it to develop a favorable environment to adapt to the acidic PH gastric environment.

Urease is very important in the pathogenesis of *Proteus mirabilis*, this enzyme is composed of UreA, UreB, UreC trimers (Jones and Mobley, 1988) and a nickel co-enzyme, which catalyzes the formation of kidney stones and bladder (Coker *et al.*, 2000; Armbruster *et al.*, 2018).

On the other hand, *Proteus mirabilis* secretes hemolysin which is cytotoxic for epithelial cells of the urinary tract (Silver and Misra, 1984). This hemolysin can induce by its toxicity a cellular disorder and cause an inflammatory state in the gastric mucosa, and this thanks to its adaptability due to its ability to secrete urease enzyme.

L2.1 isolate (anaerobic)

The detection of urease by the indole- urea test is favorable for the presence of the bacteria (Baumer *et al.*, 1992). This enzyme, an essential characteristic of *Helicobacter pylori hydrolyzes* the urea normally present in the stomach in ammonia and carbon dioxide. The ammonia released neutralizes the bacterial microenvironment by protecting it from gastric acidity (Mégraud and Lamouliatte, 1992).

The speed and intensity of the reaction depend on the time of the transfer and the bacterial density (Sobhani *et al.*, 1991) (Mégraud and Lehours, 2007; Werme *et al.*, 2015). In the case of the reaction of isolate L2,1 with indole urea, the transfer time to the pink color is obtained at 20 minutes.

similar to our results (Mégraud and Lamouliatte, 1992; Fennerty, 1994) obtained positive reactions in 20 minutes in patients suffering from a gastroduodenal disease, thus making this speed as a very favorable index in the meadow identification.

Nevertheless, about 80% of the cases studied (Delchier *et al.*, 1996) observed a positivity of urease in the first half hour.

In addition, the positive transfer of the coloration is a function of the bacterial load in the indole urea medium, that is to say depends on the number of bacteria present in the biopsy which is of the order of 105 bacteria (Megraud *et al.*, 1991; Lamouliatte, 1993; Danquechin Dorval *et al.*, 1994; De Korwin, 2003) On the other hand, the presence of *Helicobacter pylori* can not be established solely by the positivity of the indole urea test, however, the identification must be coupled by other tests (Delchier *et al.*, 1996)

Following the cytological examination of a biopsy fragment smear, the presence of a characteristic morphology specific to *Helicobacter pylori* was found (Fauchère and Rosenau, 1991). Our cytological examination confirms the presence of gram-negative, curved, V, S, comma and sometimes cocoid and slightly spiral bacilli for isolate L2,1. The presence of these forms in biopsy impressions has already been reported by (Mégraud and Lamouliatte, 1992; Mégraud, 1995; Cassel-Béraud *et al.*, 1997)

This examination demonstrates the presence of a characteristic morphology of *Helicobacter pylori* and confirms its particular location (de Mascarel *et al.*, 1989; Mégraud, 1995). At the same time, this technique has been confirmed by (de Mascarel *et al.*, 1989; Megraud *et al.*, 1991; De Korwin, 2003)

This gastric localization only confirms the presence of *Helicobacter pylori* at the level of the epithelium of this organ, highlighting the contact relations between this bacterium and the gastric epithelial cells, preferential site of *Helicobacter pylori* and confirms its membership in mucous bacteria digestive (Lamouliatte, 1993; Danquechin Dorval *et al.*, 1994; Mégraud and Lehours, 2007)

Bacteriological culture is a key tool in the identification of *Helicobacter pylori*, as well as the antibiotic susceptibility test, but it requires special attention (De Korwin, 2003)

The isolation of *Helicobacter pylori* requires a microaerobic culture (Campy-pack system) in a culture medium enriched with blood, serum or starch(Sobhani *et al.*, 1991; Fennerty, 1994; Fabre *et al.*, 1994). For this purpose, we used isolation media with added blood, such as Columbia agar, to which blood was added. This was demonstrated by the Graham team using an agar medium, enriched with 7% horse blood to treat gastric biopsies (Hachem *et al.*, 1995)

Once the bacterium was isolated, we proceeded to the study of the identification of the L2.1 isolate which is based on morphological characters (macroscopic, microscopic) and biochemical (Megraud *et al.*, 1991; Mégraud and Lamouliatte, 1992; Lamouliatte, 1993; Mégraud, 1995; Mégraud and Lehours, 2007). The particular appearance of *Helicobacter pylori* facilitates its identification (Mégraud and Lamouliatte, 1992) and these traits specify the species of *Helicobacter pylori* (Megraud, 1989; Mégraud, 1994, 1995; Mégraud and Lehours, 2007)

Microscopic observation of the L2.1 isolate revealed a Gram-negative bacterium with characteristic *Helicobacter pylori* forms. At the same time, (Mégraud, 1994) confirmed the Gram-negative nature of this bacterium. On the other hand, the existence of variable cell forms (curved, comma, C-shaped, V or S), do strengthen this particular morphology of *Helicobacter pylori* (Fauchère and Rosenau, 1991; Mégraud, 1994).

It has been found in biochemical tests that the L2.1 isolate has a positive oxidase, catalase and urease. The positivity of these three biochemical characters directed us towards a pre-identification of *Helicobacter pylori* (Megraud, 1989; Fauchère and Rosenau, 1991;

Sobhani *et al.*, 1991; Mégraud, 1994) (Perry *et al.*, 2006; Medouakh and Bensoltane a, 2011)

According to (Samuels *et al.*, 2000) the irregular distribution of bacteria in the gastric mucosa may adversely affect the appearance of colonies in culture media and may therefore contribute to false negative results. *Helicobacter pylori* (isolate, L2,1) was found to be resistant to the antibiotic disk Amoxicillin + clavulanic acid (AMC) with a diameter of the inhibition zone equal to 07 millimeters (mm).

Resistance to metronidazole and sometimes to clarithromycin poses many problems in contrast to amoxicillin and tetracycline, antibiotics which only exceptionally give resistance (De Korwin, 2003).

L3 = L3, 1 isolate (anaerobic)

This bacterium has been recognized as *Staphylococcus aureus* presenting as a hull in clusters, Gram positive and catalase positive. Its carotenoid content gives it a golden color at the origin of its name (George Y *et al.*, 2005). The species *Staphylococcus aureus* is commensal of the man, it is present in 15 to 30% of the so-called healthy carriers in whom it has a role of ecological protection, and turns out opportunistic pathogen in certain places or in certain circumstances. *Staphylococcus aureus* has pathogenic powers, including an invasive ability, ability to multiply and spread in the body, and a toxic power, ability to develop a toxin by the bacterium that has both toxic and antigenic in the host (Joël, 2017).

Staphylococcus aureus is found in about 27% of healthy individuals in the nasal cavity, in the gastrointestinal tract, and in lesser amounts on the skin and other mucous membranes. It is also found in small amounts in the digestive tract and often in the perineum (Leyden *et al.*, 1974)

Conclusions

The aim of this current work is to make a state of gastric environment concerning the bacteria isolated from gastric biopsies, co-existing with Helicobacter pylori in the case of Gastrite B.

The identification of pathogenic bacteria, including Helicobacter pylori, was carried out by methods requiring the performance of human gastric biopsies, among which we mention the rapid urease test, the cytological examination, the histological examination, culture, and the use of API Galleries.

The culture remains the reference diagnostic method because it allows on the one hand an isolation of Helicobacter pylori and identification of bacteria such as Staphylococcus aureus, Proteus-mirabilis. And on the other hand the study of antibiotic sensitivity for an effective treatment allowing the eradication of Helicobacter pylori, Staphylococcus aureus, and Proteus-mirabilis.

In parallel, the identification of these bacteria isolated from gastric biopsies was made on the basis of their morphological, biochemical characters, and API galleries. Phenotypic criteria are still important today for the classification of these isolated bacteria in research study.

Lactobacilli are known for their antibacterial effect and therefore they have the ability to protect humans against pathogenic bacteria.

In the case of our research study, lactobacilli could therefore be exploited and used as a therapeutic adjunctive agent to eradicate infection with l'Helicobacter pylori, Staphylococcus aureus, and Proteus-mirabilis identified from biopsies of patients with gastritis type B.

Acknowledgments

The authors wish to thank the Ministry of Higher Education and Scientific Research of Algeria for financial support.

Compliance with ethical standards

I respected all ethical standards

Disclosure

The authors declare no conflict of interest.

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