

Isolation and Identification of Mycoplasmas from Patients with Gastro-Intestinal Disease in Basrah Hospitals

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Abstract

The *mycoplasma* is the smallest and simplest cell with the ability of self-reproduction and is unique among prokaryotes in that it lacks a cell wall. *Mycoplasma* is similar to *Helicobacter pylori* in terms of ammonia production and the induction of inflammatory cytokines from immune and non-immune cells. The current study aims to determine the role that *Mycoplasma* bacteria play in gastrointestinal infections such as gastritis, peptic ulcers, stomach cancer and colon cancer. The study included collecting samples from (100) patients of different ages (77-9) years, males and females, who were referred to the Endoscopy Division at Al-Sadr Teaching Hospital, Basra General Teaching Hospital, Ports General Hospital and Al-Fayhaa General Hospital For the period from February 2021 to June 2021. Pathological cases were diagnosed by general specialists and abdominal surgery specilaists, as biopsy tissue biopsies were removed from the stomach and intestine by means of forceps of the endoscope device and then placed directly in a tube containing a Monophasic-Diphasic Culture Setup (MDCS) intended to isolate *Mycoplasmas*. Through the results obtained from this study it appeared that it was possible to isolate some types of *Mycoplasma* bacteria from the stomach and intestines, as 36 samples recorded a positive result out of 100 samples, and three types of *Mycoplasma* bacteria were isolated with different isolation rates. These types included the following: 17% *M. hyorhinis*, 12% *M. faucium*, and 7% *M. salivarium*. It can be concluded from the results of this study that *Mycoplasma* has an important role in gastrointestinal infections. MDCS medium is a characteristic selective medium used to isolate different types of *Mycoplasma*.

Keywords: *Mycoplasma* • Gastrointestinal diseases

Introduction

The stomach and intestine are affected by many diseases such as gastroenteritis and peptic ulcers that affect the digestive system as a result of the breakdown of the mucous layer of the gastro-intestinal tract and the gastric lymphoma adenocarcinoma of the stomach [1]. The most important reason for the occurrence of these diseases is due to the presence of some bacterial species such as *Mycoplasma* bacteria and *Helicobacter pylori* in the gastrointestinal tract [2,3]. *Mycoplasma* is a multiform organism, devoid of the cell wall, prokaryotes that exist either on eukaryotic cell membranes or inside cells, and it is the smallest organism capable of self-reproduction. It lacks the cell wall which makes it unaffected by many antibodies bioassays such as penicillin or beta-lactam that target the cell wall. This trait is also used to distinguish these bacteria from other bacteria and place them in the soft-skinned class (Mollicutes) [4,5]. These bacteria are difficult to grow and do not grow on normal culture media, and members of the *Mycoplasmataceae* family obtain amino acids, purines, pyrimidines and the rest of the components of the plasma membrane from the environment of the medium in which they grow [6]. *Mycoplasma* resembles viruses in the way they live as they are found inside the host's cells and may reach the bloodstream and spread to many tissues and organs, and due to their small size, they can pass through filter paper [7]. The *Mycoplasmataceae* family includes two genera responsible for causing disease in humans, namely the genus *Ureaplasma* and the genus *Mycoplasma* which includes several species that have been diagnosed as pathogens to humans [8]. Some types of *Mycoplasma* bacteria have special adhesive structures called the terminal tip and some of them do not have them, but rather penetrate directly into cells. Therefore, these bacteria appear as an external

pathogen as they infect the surfaces of ciliated and non-ciliated epithelial cells [9]. The main habitats of *Mycoplasma* bacteria in humans and animals are the mucous surfaces of the respiratory system, the urinary reproductive system, the gastrointestinal tract, the eyes, the joints, and the mammary glands [10]. *Mycoplasma* bacteria are found in the mouth and pharynx of humans, including *M. oral*, *M. salivarium* and *M. faucium* which produce ammonia from arginine and cause damage to host cells. *M. salivarium* is part of the oral microbial flora and inhabits gingival grooves and has the ability to play an important role in some oral infections, including periodontal diseases [11]. Some reports showed the epidemiological role of *Mycoplasma* bacteria, as these bacteria were found in patients with gastritis, gastrointestinal tumors, and gastric cancer [12,13].

Mycoplasma has an important role in causing many dangerous diseases that affect the human digestive system, as some studies have indicated the epidemiological role of *Mycoplasma* bacteria in patients with gastritis, and these bacteria are also found in people with gastrointestinal tumors, gastric cancer, and colorectal cancer [12,14].

Materials and Methods

Area of the study

The study included collecting samples from (100) patients of different ages (9-77) years, males and females, who were referred to the Endoscopy Division at Al-Sadr Teaching Hospital and Basra General Hospital for the period from February 2021 to June 2021. Medical cases were diagnosed by specialists in abdominal surgery for those who suffered from indigestion, upper abdominal pain, intestinal bleeding and vomiting. Tissue biopsies

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were taken from the antrum and intestine with forceps of the laparoscopic system. Endoscopy was performed for patients and under local anesthesia using xylocaine.

Preparing the culture media

The special Monophasic-Diphasic Culture Setup (MDCS) medium was used to isolate *Mycoplasma*

The solid phase was prepared and molded formally: Tilted in sterile 15 mm test tubes and left to cool down, then the liquid medium (liquid phase) was prepared. Liquid medium (liquid phase) and placing (1 ml) of it to cover only the lower part of the tube and thus we obtain a single-phase medium (MDCS) with the upper part remaining in a single phase [15]. The isolation medium of MDCS can be illustrated in Figure 1.

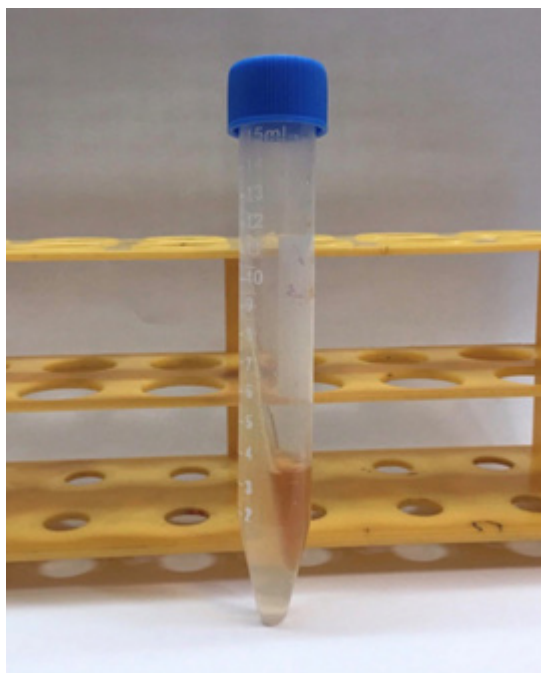


Figure 1. Shows the MDCS medium of *Mycoplasma* isolation.

Cultivation and isolation of *Mycoplasma*: The tissue biopsy was placed and injected directly into the liquid phase by moving and tilting the tube two or three times to cover the upper portion before incubation. Air incubated at 37°C for 24 hours. The colonists appeared on the sloping surface of the solid medium. After the initial isolation of the *Mycoplasma* on the mono- biphasic medium and obtaining pure cultures, biochemical tests were performed to diagnose *Mycoplasma* [16,17].

Results

Isolation and diagnosis of *Mycoplasma* from tissue biopsy specimens

The positive result of the initial isolation was revealed by changing the color of the liquid medium from red to yellow during 24-48 hours of incubation as shown in Figure 2.

Then the *Mycoplasma* colonies appeared on the solid medium after 72-24 hours of incubation where the colonies took the form of a fried egg. In this study, Three types of *Mycoplasma* bacteria were isolated from biopsy samples extracted from the stomach and intestine of patients, and *Mycoplasma* on a medium (MDCS) was diagnosed for the first time at the level of Iraq from the stomach and intestine, and these types included the following *M. hyorhinis*, *M. faucium* and *M. salivarium* . The *Mycoplasma* cells appeared spherical when stained with nigrosine stain.

Mycoplasma gave a positive result in 36 samples out of 100 samples collected from the stomach and intestines, and the isolated species appeared in different proportions. The highest isolation rate was for *M.hyorhin* is bacteria, which appeared in 17 samples out of 36 samples, and the lowest isolation percentage was for *M. salivarium* bacteria, which appeared in only 7 samples out of 36 samples, while *M. faucium* appeared in 12 samples out of 36 positive samples. '

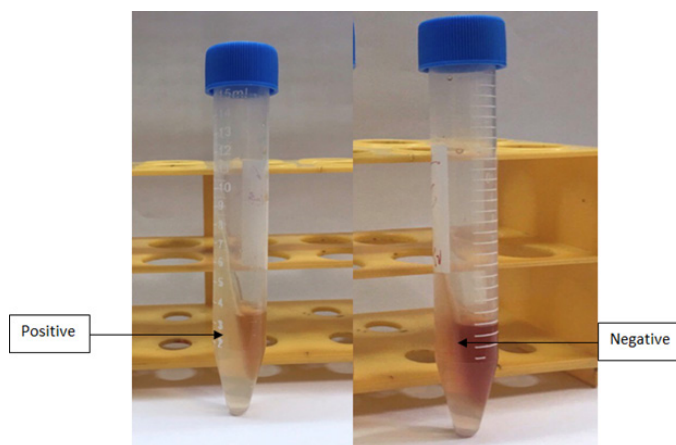


Figure 2. Shows the initial isolation of *Mycoplasma* bacteria.

Biochemical tests

In this study, the three types of isolated species were diagnosed by biochemical tests, and the following table shows the biochemical tests used

<i>Mycoplasma spp</i>	Glucose fermentation	Mannose fermentation	Arginine hydrolysis	phosphatase	Film or spot in egg yolk	Urea decomposition	Hemolysis	Tetrazolium reduction Ae/An	Hem adsorbition	Coagulated serum	Gelatin liquefaction	Casein digestion
<i>M. hyorhinis</i>	+	-	-	+	-	-	-	+/+	-	-	-	-
<i>M. faucium</i>	-	-	+	-	-	-	-	-/-	+	-	-	-
<i>M. salivarium</i>	-	-	+	-	+	-	-	-/W	-	-	-	-

An/Ae: Anaerobic/Aerobic; W: Poor

Table 1. Biochemical tests of mycoplasma isolated in this study based on Percin's evidence (Holt, et al. 1994b).

to differentiate between the isolated species (Table 1).

Discussion

A healthy digestive system is an indicator of good health in general, and the stomach as it is the home of the disease, is also the home of medicine. The stomach and intestine are affected by many diseases that occur due to eating food or drinks contaminated with bacteria or viruses. The similarities between *Mycoplasma* and *Helicobacter pylori* in the risk factors that they cause in the stomach and intestines that lead to the development of many diseases such as chronic gastritis, malignant tumors and stomach cancer encouraged us to conduct this study.

Isolation and diagnosis of *Mycoplasma* from tissue biopsy specimens

To isolate this bacterium, a Monophasic-Diphasic Culture Setup (MDCS) is used, as it is a special selective medium to isolate the *Mycoplasma* bacteria and distinguish it from the rest of the other bacterial genera. This medium is characterized by a number of unique features that distinguish it from other circles. These features are as follows:

- It requires a short incubation time, which enables rapid results to be obtained.
- The lack of use of the medium.
- The ability to identify and diagnose colonies easily and inexpensively from an economic point of view.
- As well as the use of small quantities of solid and liquid medium in one test tube
- Finally get rid of contamination.

Mycoplasma was isolated and diagnosed from biopsy samples of patients with gastrointestinal diseases. Several epidemiological studies have shown the role that *Mycoplasma* spp. in causing gastro-intestinal diseases such as gastritis, stomach ulcers, stomach cancer, duodenal ulcers, and colitis and colon cancer [18,14]. The colonies of *M. hyorhinitis*, *M. faucium* and *M. salivarium*, appeared in the form of a fried-egg which is identical to the typical colony shape characteristic of *Mycoplasma* colonies [19]. The liquid phase of the test medium turns from red to yellow according to the change in the acidic function; for the ability of *M. hyorhinitis* bacteria to ferment glucose. This is one of the important diagnostic tests for diagnosing the types of *Mycoplasma*, as this process is used to obtain energy. It promotes chronic tissue injury [20]. *M. hyorhinitis* has an important and major role in stomach and intestinal infections because it has virulence factors that help it invade tissues and remain intrusive inside or outside cells because *M. hyorhinitis* is associated with many human cancers such as stomach cancer, colorectal cancer, prostate and ovarian cancer [21,14]. *M. hyorhinitis* bacteria have large amounts of lipo-proteins, as this bacteria changes the antigen on the surface of its cell when it attaches to the host cell in order to escape from the host cell's defenses [22,23]. The *Mycoplasma* possesses N-Terminal Polypeptide terminal structures, with the help of adhesion proteins that act as *Mycoplasma* adhesion to the surface of the epithelial cells of the mucous layer of the host cells, and that the fusion of the membranes will change the composition and permeability of the cell membrane in the host cell by releasing a number of enzymes that enter the host cells. It damages DNA and affects gene expression and may lead to apoptosis [18,24].

Biochemical tests

The results of the current study showed the possibility of diagnosing *M. faucium* and *M. salivarium* bacterial species by obtaining a positive result for the L-arginine degradation test. The test medium turned from orange to pink. It was found that a gene in this bacterium encodes for the making of three enzymes necessary for the degradation of L-arginine namely, Ornithine carbamoyl transferase carbamate kinase and arginine deaminase and arginine is transformed into the compound citrulline, which

in turn is transformed through a series of reactions to ornithine and then to carbamyl phosphate to produce energy ATP and ammonia [25]. In a study conducted in Mexico, Rivera and Rodríguez indicated that the *Mycoplasma* (*M. faucium*, *M. orale* and *M. salivarium*) bacteria are similar to *H. pylori*. In terms of ammonia production that causes tissue damage and neutralizes stomach acid [26]. The results of the *Mycoplasma* diagnostic tests gave the ability of *M. faucium* bacteria to adsorb blood by surrounding colonies with red blood cells. Holt, et al. indicated that this test is an important diagnostic test [17]. Collee, et al. indicated that there is an important test in lipolysis whose results correspond to the results of the current study. It is the ability of *M. salivarium* bacteria to degrade lipolytic fats and create a transparent region surrounding the colonies of bacteria and capable of analyzing egg-yolk in the supported culture medium by adding suspension of egg yolk. The use of chicken egg yolk is a good and special medium in isolating *M. salivarium* [27]. The results of the current study showed that the rate of isolation of *Mycoplasma* bacteria was 36%, meaning that the number of positive samples was 36 out of 100 samples collected from patients of different ages and from both sexes. The most common *Mycoplasma* bacterium in biopsy samples extracted from patients with gastrointestinal diseases was *M. hyorhinitis* at 17%, and the lowest percentage was *M. salivarium* at 7%, in a study by Kwon, et al. The percentage of the bacteria of *Mycoplasma* bacteria isolation from stomach and intestinal samples was 41.1%, and the species appeared in different proportions, with the highest percentage of *M. faucium* bacteria and the lowest percentage of *M. orale* bacteria [28]. A study conducted in China by Yang, et al. was able to isolate bacteria *M. hyorhinitis* from patients with stomach cancer with an isolation rate of (39/61, 63.9%) [29].

Conclusion

Depending on the results of the current study, we conclude the following:

A *Mycoplasma* bacterium is one of the main causes of stomach and intestinal diseases. MDCS medium is a distinctive selective medium used for the first time in Iraq to isolate different types of *Mycoplasma* from the stomach and intestines.

- Isolation of different types of *Mycoplasma* bacteria (*M. hyorhinitis*, *M. faucium*, *M. salivarium*) from patients with stomach and intestinal diseases for the first time in Iraq.
- *M. hyorhinitis* is a major cause of stomach and intestinal cancer.

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