Arch. Biol. Sci., Belgrade, 64 (4), 1297-1299, 2012 DOI:10.2298/ABS1204297B

SUCCESSFUL ISOLATION OF *HELICOBACTER PYLORI* AFTER PROLONGED INCUBATION: A CASE REPORT OF PROLONGED INCUBATION FOR *H. PYLORI*

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Abstract – The culture of *Helicobacter pylori* from a gastric biopsy is the "gold standard" in the diagnosis of *H. pylori* infection. However, the primary isolation of *H. pylori* from gastric biopsies is rather demanding. The duration of incubation for the isolation of *H. pylori* has been recommended to be five to seven days: in the present case, we found that a prolonged incubation period allowed the successful isolation of *H. pylori* from a patient with ulcus ventriculi. Biopsies were placed directly into transport medium and processed for culture within two hours. On day 14, one suspected *H. pylori*-like colony appeared on one of the plates. The isolate was confirmed to be *H. pylori* based on its typical colony morphology, negative Gram stain, and positive urease, catalase and oxidase tests. The isolate, requiring 14 days recovery, later exhibited the normal growth characteristics of *H. pylori* strains, indicating its unusually long incubation requirement was a temporary predicament.

Key words: Helicobacter pylori, culture, diagnosis

INTRODUCTION

Helicobacter pylori, a gastric pathogen, is present in approximately half of the human population. It is a major causative factor of chronic gastritis and peptic ulcer disease, and is an important risk factor for the development of gastric malignancies (Marshall, 2001; Pakodi et al., 2000). Over the past years, special interest has been focused on the use of accurate non-invasive methods such as the urea breath test and the stool antigen test, but biopsy-based invasive techniques, including the rapid urease test, histology and culture, are required to confirm the infection. Culture is the most specific diagnostic method for *H. pylori* infection but its sensitivity is usually lower than that of the other methods. The isolation rates of *H. pylori* from infected individuals vary from 23.5% to 97% due to a number of factors, such as biopsy preparation, cultural environment, medium and the method adopted (Heep et al., 1999). The duration of incubation for isolation of *H. pylori* has been recommended to be five to seven days. However, in the present case, we report our observation that a prolonged incubation period of up to 14 days allowed the successful isolation of *H. pylori* from a patient with ulcus ventriculi.

CASE PRESENTATION

A patient (male, 56 years old) underwent an upper gastrointestinal endoscopy due to dyspeptic symptoms at the Center for Gastroenterology and Hepatology, Clinical Center, Niš, Serbia. The gastroenterology finding was peptic ulcer disease, and four

biopsy specimens (two from the gastric antrum and two from the corpus) were taken for rapid urease test and culture.

However, the rapid urease testing was negative from both the patient's antrum and corpus. The biopsies for microbiology testing were placed directly into transport medium at room temperature and processed for culture within two hours at the Referent Laboratory for *Helicobacter* and *Campylobacter*, Center for Microbiology, Institute for Public Health Niš. Biopsy samples were smeared on *H. pylori* selective Dent Columbia agar plates (Oxoid Ltd., London, England) supplemented with 10% horse blood (SR48; Oxoid Ltd., London, England), and incubated in a microaerophilic environment (5% O_2, 10% CO_2, and 85% N_2) using campylobacter bags (CN 25; CampyGen, Oxoid Ltd.), at 37°C. The plates were checked on days 3, 6, 8, and 10, as scheduled. However, there was no colony growing after ten days. We decided to incubate the plates further, and check the plates every day. On day 14, some suspected *H. pylori*-like colonies appeared on one of the plates from the patient's corpus. These colonies were subcultured in non-selective Columbia agar plates containing 10% horse blood in the same environment as mentioned above, for three days. The isolate was confirmed to be *H. pylori* based on its typical colony morphology, Gram-negative stain and positive urease, catalase, and oxidase tests.

DISCUSSION

Culture of the bacterium from a gastric biopsy is the "gold standard" in the confirmation of a diagnosis of *H. pylori* infection. Moreover, the isolation and identification of strains is important for the investigation of profiles of bacterial virulence, and it is essential for the determination of the drug resistance of *H. pylori*. Due to increasing resistance rates to many antibiotics, the antibiotic susceptibility determination of individual isolates is of particular importance. However, primary isolation of *H. pylori* from gastric biopsies is rather demanding, and is affected widely by number of factors such as biopsy preparation, cultural environment, medium and the method adopted. In addition, there are also biopsy related factors (Versalovic, 2003).

It was reported that a longer incubation of 11 days is helpful for isolating *H. pylori* strains from long-term frozen specimens (Boyanova, 2003), but this is the first report of the bacteria recovered after 14 days of incubation which was not previously frozen. The isolate requiring 14 days recovery later exhibited the normal growth characteristics of *H. pylori* strains when compared to the reference strain ATCC 43504 (National Collection of Type Cultures – NCTC 11637, London, UK), indicating its unusually long incubation requirement was a temporary predicament. Our report supports previous findings that a prolonged incubation is necessary for some strains, especially those exposed to detrimental environmental conditions (Nilsson et al., 2002) or a period of antibiotic force (Brenciaglia et al., 2000). It has been demonstrated *in vitro* that *H. pylori* can transform from a cultivatable spiral-shaped form to a non-cultivatable coccoid form, in which the recovery of the bacterium is very difficult by routine culture methods (Shahamat et al., 2004). It was observed that the number of coccoid forms more likely occurs in more severely damaged regions of the gastric mucosa (Saito et al., 2003). We propose that some organisms transform into the so-called "uncultivable form" under unfavorable local environments (intestinal metaplasia or antibiotic force).

The patient's result was negative for the rapid urease test, indicating that there were no organisms able to produce urease. It was shown that throughout the cycle of the coccoid form, the urease gene responsible for the production of urease was in an intact condition (Can et al., 2008; Jekti et al., 2008). However, despite the presence of urease protein in the coccoid form, the urease enzymatic activity was absent (Jekti et al., 2008). This fact has several diagnostic and clinical implications. Patients with evidence of ulcer disease and *H. pylori* infection should be treated promptly with antimicrobial therapy to eradicate *H. pylori* (Malfertheiner et al., 2007).

CONCLUSION

As culture is important in studying the profile of the antibiotic sensitivity of the isolate for the treatment of infections caused by *H. pylori*, we suggest a prolonged incubation time to obtain a higher isolation rate for *H. pylori* from clinical samples.

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