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CASE REPORT

Successful isolation of *Helicobacter pylori* after prolonged incubation from a patient with failed eradication therapy

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Abstract

Helicobacter pylori (*H pylori*), a gastric pathogen, is a major cause of chronic gastritis and peptic ulcer disease, and is an important risk factor for the development of gastric malignancies. Culture of the bacterium from gastric biopsy is essential for the determination of drug resistance of *H pylori*. However, 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. In the present case, we found that a prolonged incubation period of up to 19 d allowed successful isolation of *H pylori* from a patient who received triple therapy that failed to eradicate the bacterium.

Key words: *Helicobacter pylori*; Isolation; Eradication

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INTRODUCTION

Helicobacter pylori (*H pylori*) is a gastric pathogen, which is present in approximately half of the world's population. It is a major cause of chronic gastritis and peptic ulcer disease, and is an important risk factor for the development of gastric malignancies[1-4]. Although accurate non-invasive methods such as the urea breath test, the stool antigen test, and serology are available, biopsy-based invasive techniques, including the rapid urease test, histology and culture, are required to confirm the infection. Moreover, culture of the bacterium from gastric biopsy is essential for the determination of drug resistance of *H pylori* and thus for the subsequent treatment strategy after failed eradication therapy. However, the isolation rates of *H pylori* from infected individuals vary from 23.5% to 97%[5,6] due to a number of factors, such as biopsy preparation, cultural environment, medium and the method adopted. The duration of incubation for isolation of *H pylori* has been recommended to be 2 to 7 d. Here we reported our observation that a prolonged incubation period of up to 19 d allowed successful isolation of *H pylori* from a patient who received triple therapy that failed to eradicate the bacterium.

CASE REPORT

A patient (female, 59 years old) with an *H pylori* positive duodenal ulcer received two consecutive trials of 7-d triple regimens in a regional hospital. The regimen consisted of Metronidazole, Clarithromycin, and Cimetidine. Four weeks after the second trial, the patient was still positive for a ^{13}C urea breath test. She then came to Beijing for a solution. To obtain the drug resistance profile of the *H pylori* strain, the patient underwent an upper gastrointestinal endoscopy, and four biopsy specimens were taken from gastric antrum. The biopsies were placed directly into transport medium at room temperature and processed for culture within 2 h. Biopsy samples were smeared on *H pylori* selective Dent Columbia agar plates (Oxoid Ltd., London, England) supplemented with 8% sheep blood (Hengzhaoxiang Science & Technology Co., Beijing), and incubated in a microaerophilic environment (5% O_2, 10% CO_2, and 85% N_2) at 37℃. The plates were scheduled to be checked on days 3, 6, 8, and 10. However, there was no colony growing after 10 d. We decided to incubate the plates further, and check the plates every 3 d. On day 19, one suspected *H pylori*-like colony appeared

on one of the plates. This colony was subcultured in non-selective 2 Columbia agar plates containing 8% sheep blood in the same environment mentioned above, for three days. The isolate was confirmed to be *H pylori* based on its typical colony morphology, negative Gram's stain, and positive urease, catalase, and oxidase tests.

The antimicrobial susceptibilities of the isolate to metronidazole, clarithromycin, amoxicillin, ampicillin, levofloxacin, and rifampicin were determined by the E-test (AB Biodisk, Solna, Sweden). Briefly, a bacterial suspension (2 McFarland standard) was prepared with brain heart infusion (Oxoid Ltd., London, England) containing 10% heat-treated serum[7]. After the bacterial suspension was swabbed onto the entire Columbia plates, sterile E-test strips impregnated with the above antibiotics were placed on the agar surface of corresponding plates. Minimal inhibitory concentraction (MICs) were determined according to the manufacturer' s instructions after three to four days of incubation. The isolate exhibited high-level resistance to metronidazole (MIC > 256 μg/mL) and clarithromycin (MIC > 48 μg/mL), but was susceptible to amoxicillin, ampicillin, levofloxacin, and rifampicin (all MIC < 0.016 μg/mL), which explained the failure of the triple regimens containing metronidazole and clarithromycin.

DISCUSSION

Culture has been considered the "gold standard" in confirmation of the diagnosis of *H pylori* infection. Moreover, the isolation and identification of strains is important for the investigation of profiles of bacterial virulence and, particularly, drug resistance. Due to the gradually rising prevalence of *H pylori* resistance to many antibiotics commonly used in triple regimens, the determination of antibiotic susceptibility of individual isolates is of particular importance. However, primary isolation of *H pylori* from gastric biopsies is rather demanding, and is affected widely by the culture conditions in addition to the biopsy-related factors[8]. Our report also indicates that a prolonged incubation is necessary for some strains, especially those enduring hostile environment or a period of antibiotic force. It was reported that a longer incubation of 11 d is helpful for isolating *H pylori* strains from long-term-frozen specimens[9], but this is the first report of the bacteria recovered after 19 d incubation. Indeed, the isolate requiring 19 d recovery later exhibited normal growth characteristics of *H pylori* strains when compared to another strain, NCTC11637, indicating its unusually long incubation requirement was a temporary predicament.

It has been demonstrated *in vitro* that *H pylori* cells 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[10]. We would propose that during the period of eradication therapy, some organisms transform into the so-called "uncultivatable form" with the propagation being stopped under the antimicrobial pressure in the local environment. However, these organisms, which may have been selectively resistant to the used antimicrobials, survive, possibly with some suppressed metabolic activities[11]. Once released from the medication at the end of the trial, these organisms gradually restore their normal growing features after prolonged incubation in an optimal environment and eventually become cultivatable. Therefore, these "uncultivatable form" organisms might contribute, at least partially, to treatment failures and the development of antimicrobial resistance. In the meantime, we suggest that a new *H pylori* culture after a first attempt to eradicate *H pylori* needs to be postponed, probably by four weeks or even longer. It is noticeable that the patient was positive for the ^{13}C urea breath test four weeks after completion of treatment, indicating that there are a number of organisms that are able to produce urease activities after release from antimicrobial pressure after four weeks. The coccoid *H pylori* can produce urease, though at a decreased level[12], suggesting its potential pathogenicity.

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