**Name of journal: World Journal of Gastroenterology**

**ESPS Manuscript NO: 11987**

**Columns: CLINICAL TRIALS STUDY**

**Centralized isolation of *Helicobacter pylori* in a large number of clinical samples from multiple centers and the influences of transport conditions**

Gong YN *et al*. Centralized isolation of *H. pylori*

Ya-Nan Gong,You-Ming Li, Ning-Min Yang, Hong-Zhang Li, Feng Guo, Lang Lin, Qun-Ying Wang, Jia-Kun Zhang, Zi-Zhong Ji, Ji-Bo Mao, Jun-Liang Mao, Zheng-Chao Shi, Wu-Heng Tang, Xin-Jian Zhu, Wei Shao, Xiao-Feng Zhang, Xing-Hua Wang, Yue-Feng Tong, Mi-Zu Jiang, Guang-Lan Chen, Zhi-Yong Wang, Hui-Min Tu, Guo-Fa Jiang, Jian-Sheng Wu, Xu-Peng Chen, Qiu-Long Ding, Hong Ouyang, Feng-Zhe Jin, Yan-Li Xu, Jian-Zhong Zhang

**Ya-Nan Gong, Jian-Zhong Zhang,** State Key Laboratory for Infectious Disease Prevention and Control, National Institute for Communicable Disease Control and Prevention, Chinese Center for Disease Control and Prevention, Beijing 102206, China

**Ya-Nan Gong, Jian-Zhong Zhang,** Collaborative Innovation Center for Diagnosis and Treatment of Infectious Diseases, Hangzhou 310003, Zhejiang Province, China

**You-Ming Li**, the First Affiliated Hospital, College of Medicine, Zhejiang University, Hangzhou 310003, Zhejiang Province, China

**Ning-Min Yang**, Zhiyuan Medical Inspection Institute CO., LTD, Hangzhou 310021, Zhejiang Province, China

**Hong-Zhang Li,** Sanmen People’s Hospital, Taizhou 317100, Zhejiang Province, China

**Feng Guo**, the First People’s Hospital of Xiaoshan District, Hangzhou 311200, Zhejiang Province, China

**Lang Lin**, the First People’s Hospital of Cangnan, Wenzhou 325800, Zhejiang Province, China

**Qun-Ying Wang,** Jinhua Municipal Central Hospital, Jinhua 321001, Zhejiang Province, China

**Jia-Kun Zhang,** the First People’s Hospital of Pingyang, Wenzhou 325400, Zhejiang Province, China

**Zi-Zhong Ji,** the First Hospital of Jiaxing, Jiaxing 314001, Zhejiang Province, China

**Ji-Bo Mao,** Zhoushan Hospital, Zhoushan 316021, Zhejiang Province, China

**Jun-Liang Mao,** the First People’s Hospital of Wenling, Wenling 317500, Zhejiang Province, China

**Zheng-Chao Shi,** Rui’an People’s Hospital, Rui’an 325200, Zhejiang Province, China

**Wu-Heng Tang,** Maternal and Child Health Hospital of Zhoushan City, Zhoushan 316000, Zhejiang Province, China

**Xin-Jian Zhu,** Shangyu People’s Hospital, Shaoxing 312300, Zhejiang Province, China

**Wei Shao,** People’s Hospital of Putuo District, Zhoushan 316399, Zhejiang Province, China

**Xiao-Feng Zhang,** Hangzhou First People’s Hospital, Hangzhou 310006, Zhejiang Province, China

**Xing-Hua Wang,** Qingtian People’s Hospital, Lishui 323900, Zhejiang Province, China

**Yue-Feng Tong,** the First People’s Hospital of Yongkang, Yongkang 321300, Zhejiang Province, China

**Mi-Zu Jiang,** the Children’s Hospital Zhejiang University School of Medicine, Hangzhou 310003, Zhejiang Province, China

**Guang-Lan Chen,** Lishui People’s Hospital, Lishui 323000, Zhejiang Province, China

**Zhi-Yong Wang,** The Affiliated Hospital of Hangzhou normal university, Hangzhou 310015, Zhejiang Province, China

**Hui-Min Tu,** Wuxi No 4 Hospital Affiliated to Suzhou University, Wuxi 214062, Zhejiang Province, China

**Guo-Fa Jiang,** Jinhua Wenrong Hospital, Jinhua 321001, Zhejiang Province, China

**Jian-Sheng Wu,** the First Affiliated Hospital of Wenzhou Medical College, Wenzhou 325000, Zhejiang Province, China

**Xu-Peng Chen,** Yueqing People’s Hospital, Wenzhou 325600, Zhejiang Province, China

**Qiu-Long Ding,** Tiantai People’s Hospital, Taizhou 317200, Zhejiang Province, China

**Hong Ouyang,** Lin'an people's Hospital, Lin'an 311300, Zhejiang Province, China

**Feng-Zhe Jin,** Rui’an City TCM Hospital, Rui’an 325200, Zhejiang Province, China

**Yan-Li Xu,** Medical College of Hebei University of Engineering, Handan 056038, Hebei Province, China

**Author contributions:** Gong YN and Xu YL analyzed data and drafted this paper; Li YM and Yang NM were involved in designing protocol and providing results of this study; Zhang JZ had the idea for this study and had final responsibility for the decision to submit for publication; all the other authors were responsible for sample collection, the order of authorship was based on specific contribution; all authors were involved in data interpretation and critical revisions of the report, and approved the final version.

**Supported by** Science and Technology Program of Zhejiang Province, China No. 2001C23140; the grant from National Technology RD Program in the 12th Five-Year Plan of China No. 2012BAI06B02; the Major Technology Project as part of "Prevention and Control of Major Infectious Diseases including AIDS and Viral Hepatitis" No. 2013ZX10004216-002; the grant from National Key Scientific Instrument and Equipment Development Project No. 2012YQ180117; Medical and Health Science and Technology Plan Project of Zhejiang Province No. 2012KYB248; and the Science and Technology Project of Zhejiang province No. 2011C23140

**Correspondence to: Jian-Zhong Zhang,** **MD, PhD,** State Key Laboratory for Infectious Disease Prevention and Control, National Institute for Communicable Disease Control and Prevention, Chinese Center for Disease Control and Prevention, 155 Changbai Road, Changping District, Beijing 102206, China. [zhangjianzhong@icdc.cn](mailto:zhangjianzhong@icdc.cn)

**Telephone:** +86-10-58900707 **Fax:** +86-10-58900700

**Received:** June 16, 2014 **Revised:** August 11, 2014

**Accepted:** September 18, 2014

**Published online:**

**Abstract**

**AIM:** To evaluate the efficacy of centralized culture and possible influencing factors.

**METHODS:** From January 2010 to July 2012, 66452 patients [with suspected *Helicobacter pylori* (*H. pylori*) infection] from 26 hospitals in Zhejiang Province and Jiangsu Province, China, underwent gastrointestinal endoscopy. The gastric mucosal biopsies were taken from the antrum for culture. These biopsies were transported under natural environmental temperature to the central laboratory in Hangzhou city and divided into three groups based on their transport time (independent 5 h, 24 h and 48 h transport groups). The culture results were reported after 72 h and the culture positive rates were analyzed by *χ*2 test. Another 5736 biopsies from *H. pylori* positive[5646 rapid urease test (RUT) positive and 90 14C-urease breath test (14C-UBT) positive] patients were also cultured as quality control in the central laboratory setting.

**RESULTS:** The culture positive rate was 31.66% (21036/66452) of the 66452 specimens and 71.72% (4114/5736) of the *H. pylori* positive specimens which were detected by RUT and 14C-UBT. In 5 h transport group, the culture positive rate was 30.99% (3865/12471) while it was 32.84% (14960/45553) in 24 h transport group. In contrast, the culture positive rate declined significantly in 48 h transport group (26.25%, *P* < 0.001). During transportation, the average natural temperature increased from 4.67℃ to 29.14℃ while the culture positive rate declined from 36.67% (1462/3987) to 24.12% (1799/7459). When the temperature exceeded 24℃, the culture positive rate decreased significantly, especially in 48 h transport group (23.17%).

**CONCLUTION:** Transportation of specimens within 24 h and under a low natural temperature (not exceeding 24℃) is reasonable and acceptable, indicating the centralized culture of multicenter samples is feasible. The results of this study can be promisingly applied in individualized medical use and clinical *H. pylori* eradication.

© 2014 Baishideng Publishing Group Inc. All rights reserved.

**Key words:** *Helicobacter pylori*;Centralized isolation; Multiple centers; Personalized treatment; Influencing factor

**Core tip:** The centralized culture of *Helicobacter pylori* (*H. pylori*) in a large number of clinical samples from multiple centers was established. The effiency of centralized culture and possible influencing factors were evaluated. Our study was the first large-scale one on centralized culture of *H. pylori*, confirming the feasibility of establishing a culture center for individualized medical use. The findings of this study can be promisingly applied in clinical and public health practice.

Gong YN, Li YM, Yang NM, Li HZ, Guo F, Lin L, Wang QY, Zhang JK, Ji ZZ, Mao JB, Mao JL, Shi ZC, Tang WH, Zhu XJ, Shao W, Zhang XF, Wang XH, Tong YF, Jiang MZ, Chen GL, Wang ZY, Tu HM, Jiang GF, Wu JS, Chen XP, Ding QL, Ouyang H, Jin FZ, Xu YL, Zhang JZ. Centralized isolation of Helicobacter pylori in a large number of clinical samples from multiple centers and the influences of transport conditions. *World J Gastroenterol* 2014; In press

**INTRODUCTION**

*Helicobacter pylori (H. pylori)*, one of the most common human pathogens, can lead to gastric ulcer, gastritis, gastric cancer, and mucosa-associated lymphoid tumors (MALT)[1].According to Maastricht IV and Chinese Consensus Report, two antibiotics combined with one proton-pump inhibitor (PPI) are recommended as standard first-line treatment to eradicate *H. pylori*[2,3]. However in recent years, the success rates have declined below 80% in most European and Asian countries[4-6]. There are many reasons accounting for eradication failure, such as poor patient compliance, resistant bacteria and low gastric pH. In fact, the main reason is the increasing *H. pylori* resistance to the antibiotics used[7,8].

In China, the resistance to clarithromycin, a key antibiotic in the triple therapy, has reached above 20% and should not be used in anti-*H.pylori* therapy without a susceptibility test[2,9,10,11]. In addition, accumulated evidences have suggested that culture-susceptibility test may improve the eradication rate of *H. pylori*[12-17].

However, *H*. *pylori* is a rather fastidious bacterium at culture, especially when a low bacterial load is present[18].The ideal situation for culture-susceptibility test is not available in many clinical settings, since most endoscopic units do not have any direct access to a microbiology laboratory. Therefore, the gastric biopsy specimens from multicenter should be transported to a central laboratory for *H*. *pylori* culture. During transportation, time and temperature could influence the survivability of *H. pylori* and these issues are still in debate[19-23]. Some investigators emphasized the need for rapid transport at a low temperature[21]. Others demonstrated that *H. pylori* could survive at room temperature for 24 h without loss of the ability to recover[22]. If as a routine clinical application system, the transport of samples under natural temperature is demanded. In order to assess the factors, transportation test[24] and isolation test of thousands of *H. pylori* strains were performed in our lab, but the complexity of real large-scale clinical application couldn’t be represented accurately. The feasibility of centralized isolation of *H. pylori* in a large number of clinical samples from multicenter and its influencing factors need to be tested for real practice.

A central laboratory for *H*. *pylori* isolation was set up in Zhiyuan Medical Inspection Institute CO., LTD in Hangzhou city which provided personalized treatment strategy for *H*. *pylori* eradication recent years. In order to evaluate the efficacy of centralized culture and possible influencing factors, we conducted a research in the central laboratory to analyze culture positive rates of a large number of clinical samples collected from 26 hospitals in nine cities.

**MATERIALS AND METHODS**

***Sample collection***

The sampling was conducted in Zhejiang Province and Jiangsu Province, China. Between January 2010 and July 2012, consecutive participants with suspected *H. pylori* infection underwent gastrointestinal endoscopy. The gastric mucosal biopsies were taken from the greater curvature of gastric antrum using sterile disposable biopsy forceps. Additionally, in order to assess the positive rate of *H. pylori* culture, specimens from *H. pylori* positive patients[5646 rapid urease test (RUT) positive and 90 14C-urease breath test (14C-UBT) positive] were also collected as control. The *H*. pylori positive samples were all collected from Wenzhou city, including The First Affiliated Hospital of Wenzhou Medical College and The First People’s Hospital of Pingyang.

This study was approved by the Ethics Committee of **National Institute for Communicable Disease Control and Prevention,** Chinese Center for Disease Control and Prevention and the Ethics Committee of Zhiyuan Medical Inspection Institute CO., LTD in Hangzhou, and written informed consent was obtained from all patients.

***Transport and culture procedure***

The gastric mucosal sample from each patient was stored in sterile tube that contained 1ml brain heart infusion (BHI) with 20% glycerol and kept under 4 ℃ before transportation. All the fresh biopsy specimens were transported under external temperature to Zhiyuan Medical Inspection Institute CO., LTD in Hangzhou for centralized isolation. The 66452 specimens were divided into three independent groups based on transport time: 5 h, 24 h and 48 h transport groups. The first group (5 h group, 12471 cases) was collected from Hangzhou city at 9 am to 12 am and cultured at 2 pm on the same day. The second group (24h group, 45553 cases) was collected from other cities hundreds of kilometers away from Hangzhou at 9 am to 12 am and sent to Hangzhou for culture at 9 am next day. The third group (48 h transport group, 8428 cases) were collected from Jinhua city and transported to Hangzhou for culture 48 hours later because of the limitations of local hospital conditions. All samples were kept under 4 ℃ before transportation. Moreover, the *H*. pylori positive samples were transported as 24 h group.

These samples were grinding broken and cultivated on Columbia agar (Oxoid) plates supplemented with 5% defibrinated sheep blood, 3 μg/mL synergist, 2.5 μg/mL vancomycin, 2 μg/mL amphotericin B, and 2μg/ml bacillosporin B under microaerophilic conditions (5% O2, 10% CO2, 85% N2) for 72 h. Translucent colonies about 0.5-2 mm from the original agar plates were selected for gram stain and for urease, oxidase and catalase tests. Colonies with curved gram-negative rods resembling *Helicobacter.spp* and positive in three enzyme tests were identified as *H. pylori*.

***Meteorological data***

The information concerning daily measured maximum, minimum and average temperatures in each city related with this study in Zhejiang Province from January 1, 2010 to December 31, 2012 was supplied by Meteorological Bureau of Zhejiang Province. The daily average temperature was the average value of temperatures at four time points each day (2 am, 8 am, 2 pm and 8 pm). In our investigation, we used the meteorological data on regional level in six cities (Hangzhou, Jiaxing, Jinhua, Taizhou, Wenzhou, and Zhoushan) to calculate the monthly maximum, minimum and average temperatures and draw the temperature change curves.

***Statistical analysis***

The results of isolation and identification of *H. pylori* for all specimens were reported 72 h after culture and all calculations were done by using the software package SPSS 18.0(SPSS Inc., Chicago, IL). The culture positive rates were assessed with *χ*2 test. A probability (*P*) value equal to or less than 0.05 was considered to be statistically signiﬁcant.

**RESULTS**

***Sample collection***

The random 66452 samples were collected from 24 hospitals in nine cities: Hangzhou (6), Jiaxing (1), Jinhua (3), Shaoxing (1), Taizhou(3), Wenzhou (4), Zhoushan (3), Lishui (2) and Wuxi (1). The 5736 *H. pylori* positive specimens were collected from another two hospitals in Wenzhou city. Eight of the all 26 sampling hospitals are Class 2A hospitals, which provide medical services for communities and majority are County-level hospitals. The others belong to Class 3A which are regional hospitals and possess the highest medical level in Chinese hospital grading. The distribution and numbers of samples in each year and city are shown in Figure 1A.

***Culture results***

Of the 66452 specimens, 21036 (31.66%，21036/66452) were *H*. *pylori* cultural positive. The culture positive rates of specimens in 2010, 2011 and 2012 were 28.64% (4897/17098), 30.28% (6513/21509) and 34.57% (9626/27845), respectively. The differences showed statistical significance (*P* < 0.001). The culture positive rate for Class 2A hospitals was 31.96% (8103/25350) and that of Class 3A hospitals was 31.47% (12933/41102).

As Figure 1B displayed, the culture positive rates of samples from nine cities varied from 26.59% (2354/8852) in Jinhua city to 42.51% (332/781) in Lishui city. The culture positive rate of Hangzhou city, which was the culture center, was 30.99% (3866/12473).

Even in the same city, the culture positive rates of different sampling hospitals were different. We compared the culture positive rates of four hospitals in Hangzhou city, reaching the culture positive rates of 29.97% (3316/11064), 38.95% (298/705), 25.73% (79/307) and 47.66% (61/128), respectively (shown in Figure 2).

***Culture results of different transport groups***

The culture positive rates of 5 h group, 24 h group and 48 h transport group were 30.99% (3865/12471), 32.84% (14960/45553) and 26.25% (2211/8428), respectively. The culture positive rate of 48 h transport group was much lower than the other two groups (*P* < 0.001). We also compared the culture positive rate of 48 h transport group with that of samples from same city, which were identified as 24 h transport group. The results showed significant difference between them (26.25% *vs* 33.73% (143/424), *P* < 0.001).

***Culture results of different natural transport temperatures***

The average natural temperature in Zhejiang province within one year was from 4.67 ℃ to 29.14 ℃. The average temperatures in January, May, June and July were 4.66℃, 20.85℃, 24.24℃ and 28.85℃, respectively. The change curves of average temperatures were similar in six cities, and the monthly average temperature, average maximum and minimum temperatures of the six cities during this study were showed in Figure 3A.

As the temperature increased, the total culture positive rate declined from 36.67% (1462/3987) in December to 24.12% (1799/7459) in July. Although the average temperature elevated significantly from January to May, the culture positive rates were similar. When the average temperature exceeded 24℃ in June and July, the culture positive rates declined significantly (*P* < 0.001), especially in July with the lowest culture positive rate (24.12%, 1799/7459). This phenomenon was particularly evident in Wenzhou city whose culture positive rates changed from 38.44% (316/822) in January to 25.38% (389/1533) in July.

The culture positive rates in the other months changed slightly even when the temperatures changed significantly (Figure 3B).

***Culture results of H. pylori positive specimens***

Of the*H. pylori* positive specimens, the isolation of *H. pylori* was successful in 4114 cases (71.72%). It indicated that the positive predictive value of *H. pylori* isolation in the central cultural platform was 71.72%. According to this data, the *H. pylori* infectious rate in the patients of random sample group was approximately 44%.

**DISCUSSION**

Isolation of all samples was conducted in the same laboratory to eliminate the intrinsic variability in multiple laboratories. The advantage of our study was its largest sample size to date, involving wide range of demographic characteristics, geographic areas and hospitals at different medical levels (as nearly all the gastrointestinal endoscopic tests were done in the Class 2A and Class 3A hospitals in China). Compared with previous studies[25], the limitations of small sample size or the data from different laboratories were certainly avoided in this study and more reliable information for the centralized culture of *H. pylori* was provided.

The culture positive rate of *H. pylori* positive specimens was 71.72%. In the previous studies, estimated isolation rates ranging from 75% to 94% were reported[25,26].Considering the differences between the *H. pylori* cultures used for clinic study and those for individual medical service, as well as on the amount of samples and the Time-efficient characteristics (a limited time of about seven days for clinical report was usually set in the latter), the culture positive rate of 71.72% for positive samples was acceptable.

In China, the total infectious rate of *H. pylori* was relatively high, reaching 40%-60% in adults. In this study, the culture positive rate of the suspected *H. pylori* infection group was 31.66%. Combined with the culture rate of positive samples, an approximate 44% infectious rate was calculated and agreed with the reported data, indicating the culture positive rate of random samples was reasonable. In addition, the cost of culture and sensitivity test for six antibiotics is totally $18, while the UBT test price is $29 in Zhejiang province. The random samples were collected without any *H. pylori* detection, leading to a lower cost (51.25% reduced) and economic burden in this population compared with detection strategy.

Factors influencing the isolation rates during transportation have been discussed by previous studies[18,23,27,28]. Use of dry ice and need for storage at a constant temperature of 4℃ were recommended, but they cannot be put into real practices in the *H. pylori* isolation for individualized medical need in a large number of cases, because dry ice is too costly and constant 4℃ preservation condition is hard to maintain. As repeated freezing and thawing was more harmful for the successful isolation of *H. pylori,* it was not recommended that the biopsy samples were transported on ice, even it appears as necessary conditions for the optimal diagnostic of Helicobacter infections by mean of bacteriological methods in small sample study[29]. Thus, in our study, transportation of biopsy specimens under ambient temperature was performed. Our results indicated that the samples could be maintained well without influencing the culture rates within 24 h, especially when the mean temperature was lower than 24℃. A prolonged or delayed transport time and the higher natural temperature (daily average temperature above 24℃) could influence the culture positive rates significantly, probably due to contamination and overgrowth of other bacteria. Based on these factors, the proper position of the culture center should be taken into account and transportation condition of low temperature measure should be taken in summer.

Besides the two strong influencing factors, other factors such as sampling and operation levels should also be considered, since variability in the culture positive rates was observed among the four hospitals in Hangzhou city which shared the same sample population and transport conditions (Figures 2 and 4). In some study[30], low isolation rates were got in failed eradication therapy patients. The patients from Class 2A hospitals and Class 3A hospitals might have different background of antibiotics use. In this study, a novel design was to compare the culture rates between Class 2A and Class 3A hospitals. The results showed no obvious differences, indicating the medical levels of hospitals could be ignored. In addition, the culture positive rates significantly increased within three years, probably due to the improved skills of sampling and experience in the laboratory personnel. This indicated that the culture positive rate also has a large space to improve, even in *H. pylori* isolation platform for individualized medical need in a large number of cases.

There were also some limitations in this study. First, in this study we further compared the culture positive rates of only four hospitals in Hangzhou city. The other two hospitals were excluded in the results, since the samples in the two hospitals were small and the sampling was not consecutive. Second, we selected six cities to assess the temperature effects on culture positive rates，because the samples collected in Lishui, Wuxi and Shaoxing for some months were missed, and the meteorological data was incomplete. Even these exclusions existed, the selected data accounted for majority and was enough to compare these differences. Third, the *H. pylori* positive group was available to assess the culture results, although there was no direct comparison or control group in this study for all samples, such as a non-culture based test, the culture positive rates in this study were considered acceptably.

In general, this study was the first large-scale one on centralized culture of *H. pylori*, confirming the feasibility of establishing a culture center for individualized medical use. We believe the findings of this study can be promisingly applied in clinical and public health practice. The resistance to clarithromycin often poses a challenge in clinical design making. Like ten years ago, when triple therapy was recommended since the eradication rate of two couplet therapeutics decreased, the doctors had to choose the quadruple therapy or sequential therapy. But this is just entering the next cycle, the eradication rate of quadruple therapy or sequential therapy will be reduced to unacceptable levels. Establishment of a personalized treatment strategy may potentially resolve the public health problem and reduce economic burden on the patients and [community](http://dict.baidu.com/s?wd=community), especially in those populations with high resistance.

**COMMENTS**

***Background***

The centralized culture of *Helicobacter pylori* (*H. pylori*) in a large number of clinical samples from multiple centers was established. The effiency of centralized culture and possible influencing factors was evaluated.

***Research frontiers***

The antibiotic resistance is a worldwide problem that prevents the *H. pylori* eradication. Personalized treatment strategy based on culture and antimicrobial susceptibility test is one of the most promising ways to solve the problem.

***Innovations and breakthroughs***

Previous studies had discussed the factors influencing the isolation rates during transportation. Our study was the first large-scale one on centralized culture of *H. pylori*, confirming the feasibility of establishing a culture center for individualized medical use.

***Applications***

The findings of this study can be promisingly applied in clinical and public health practice. Establishment of a personalized treatment strategy may potentially resolve the public health problem and reduce economic burden on the patients and [community](http://dict.baidu.com/s?wd=community), especially in those populations with high resistance.

***Terminology***

Clarithromycin is one of the core antibiotics of triple regimen for *H. pylori* eradication. If the clarithromycin resistance rate of *H. pylori* is up to 15-20% in a population, this antibiotic should not be used without susceptibility test.

***Peer review***

In this study, the authors evaluated the effiency of centralized culture and disscussed the possible influencing factors. It revealed that centralized culture of multicenter samples is feasible. The results of this study can be promisingly applied in individualized medical use and clinical *H. pylori* eradication.

**REFERENCES**

1 **Parsonnet J**. Helicobacter pylori: the size of the problem. *Gut* 1998; **43** Suppl 1: S6-S9 [PMID: 9764031 DOI: 10.1136/gut.43.2008.S6]

2 **Malfertheiner P**, Megraud F, O'Morain CA, Atherton J, Axon AT, Bazzoli F, Gensini GF, Gisbert JP, Graham DY, Rokkas T, El-Omar EM, Kuipers EJ. Management of Helicobacter pylori infection--the Maastricht IV/ Florence Consensus Report. *Gut* 2012; **61**: 646-664 [PMID: 22491499 DOI: 10.1136/gutjnl-2012-302084]

3 **Hu FL**, Hu PJ, Liu WZ, De Wang J, Lv NH, Xiao SD, Zhang WD, Cheng H, Xie Y. Third Chinese National Consensus Report on the management of Helicobacter pylori infection. *J Dig Dis* 2008; **9**: 178-184 [PMID: 18956598 DOI: 10.1111/j.1751-2980.2008.00342.x]

4 **Altintas E**, Sezgin O, Ulu O, Aydin O, Camdeviren H. Maastricht II treatment scheme and efficacy of different proton pump inhibitors in eradicating Helicobacter pylori. *World J Gastroenterol* 2004; **10**: 1656-1658 [PMID: 15162544]

5 **Gumurdulu Y**, Serin E, Ozer B, Kayaselcuk F, Ozsahin K, Cosar AM, Gursoy M, Gur G, Yilmaz U, Boyacioglu S. Low eradication rate of Helicobacter pylori with triple 7-14 days and quadriple therapy in Turkey. *World J Gastroenterol* 2004; **10**: 668-671 [PMID: 14991935]

6 **Suzuki H**, Nishizawa T, Hibi T. Helicobacter pylori eradication therapy. *Future Microbiol* 2010; **5**: 639-648 [PMID: 20353303 DOI: 10.2217/fmb.10.25]

7 **Mégraud F**, Lamouliatte H. Review article: the treatment of refractory Helicobacter pylori infection. *Aliment Pharmacol Ther* 2003; **17**: 1333-1343 [PMID: 12786627 DOI: 10.1046/j.1365-2036.2003.01592.x]

8 **Mégraud F**. H pylori antibiotic resistance: prevalence, importance, and advances in testing. *Gut* 2004; **53**: 1374-1384 [PMID: 15306603 DOI: 10.1136/gut.2003.022111]

9 **Gao W**, Cheng H, Hu F, Li J, Wang L, Yang G, Xu L, Zheng X. The evolution of Helicobacter pylori antibiotics resistance over 10 years in Beijing, China. *Helicobacter* 2010; **15**: 460-466 [PMID: 21083752 DOI: 10.1111/j.1523-5378.2010.00788.x]

10 **Su P**, Li Y, Li H, Zhang J, Lin L, Wang Q, Guo F, Ji Z, Mao J, Tang W, Shi Z, Shao W, Mao J, Zhu X, Zhang X, Tong Y, Tu H, Jiang M, Wang Z, Jin F, Yang N, Zhang J. Antibiotic resistance of Helicobacter pylori isolated in the Southeast Coastal Region of China. *Helicobacter* 2013; **18**: 274-279 [PMID: 23418857 DOI: 10.1111/hel.12046]

11 **Malfertheiner P**, Megraud F, O'Morain C, Bazzoli F, El-Omar E, Graham D, Hunt R, Rokkas T, Vakil N, Kuipers EJ. Current concepts in the management of Helicobacter pylori infection: the Maastricht III Consensus Report. *Gut* 2007; **56**: 772-781 [PMID: 17170018 DOI: 10.1136/gut.2006.101634]

12 **Dore MP**, Leandro G, Realdi G, Sepulveda AR, Graham DY. Effect of pretreatment antibiotic resistance to metronidazole and clarithromycin on outcome of Helicobacter pylori therapy: a meta-analytical approach. *Dig Dis Sci* 2000; **45**: 68-76 [PMID: 10695616 DOI: 10.1023/A: 1005457226341]

13 **Realdi G**, Dore MP, Piana A, Atzei A, Carta M, Cugia L, Manca A, Are BM, Massarelli G, Mura I, Maida A, Graham DY. Pretreatment antibiotic resistance in Helicobacter pylori infection: results of three randomized controlled studies. *Helicobacter* 1999; **4**: 106-112 [PMID: 10382124 DOI: 10.1046/j.1523-5378.1999.99002.x]

14 **Glupczynski Y**, Mégraud F, Lopez-Brea M, Andersen LP. European multicentre survey of in vitro antimicrobial resistance in Helicobacter pylori. *Eur J Clin Microbiol Infect Dis* 2001; **20**: 820-823 [PMID: 11783701 DOI: 10.1007/s100960100611]

15 **Wang G,** Zhao Q, Li S. Study of drug sensitivity test in Helicobacter pylori eradication therapy. *J Clin Intern Med* 2008; **25**: 474–477

16 **Romano M**, Iovene MR, Montella F, Vitale LM, De Simone T, Del Vecchio Blanco C. Pretreatment antimicrobial-susceptibility testing in the eradication of H. pylori infection. *Am J Gastroenterol* 2000; **95**: 3317-3318 [PMID: 11095372 DOI: 10.1111/j.1572-0241.2000.03317.x]

17 **Romano M**, Marmo R, Cuomo A, De Simone T, Mucherino C, Iovene MR, Montella F, Tufano MA, Del Vecchio Blanco C, Nardone G. Pretreatment antimicrobial susceptibility testing is cost saving in the eradication of Helicobacter pylori. *Clin Gastroenterol Hepatol* 2003; **1**: 273-278 [PMID: 15017668 DOI: 10.1016/S1542-3565(03)00131-9]

18 **Soltesz V**, Zeeberg B, Wadström T. Optimal survival of Helicobacter pylori under various transport conditions. *J Clin Microbiol* 1992; **30**: 1453-1456 [PMID: 1624562]

19 **Yuen B**, Zbinden R, Fried M, Bauerfeind P, Bernardi M. Cultural recovery and determination of antimicrobial susceptibility in Helicobacter pylori by using commercial transport and isolation media. *Infection* 2005; **33**: 77-81 [PMID: 15827875 DOI: 10.1007/s15010-005-4071-y]

20 **Xia HX**, Keane CT, O'Morain CA. Determination of the optimal transport system for Helicobacter pylori cultures. *J Med Microbiol* 1993; **39**: 334-337 [PMID: 8246249 DOI: 10.1099/00222615-39-5-334]

21 **Roosendaal R**, Kuipers EJ, Peña AS, de Graaff J. Recovery of Helicobacter pylori from gastric biopsy specimens is not dependent on the transport medium used. *J Clin Microbiol* 1995; **33**: 2798-2800 [PMID: 8567932]

22 **Heep M**, Scheibl K, Degrell A, Lehn N. Transport and storage of fresh and frozen gastric biopsy specimens for optimal recovery of Helicobacter pylori. *J Clin Microbiol* 1999; **37**: 3764-3766 [PMID: 10523597]

23 **Siu LK**, Leung WK, Cheng AF, Sung JY, Ling TK, Ling JM, Ng EK, Lau JY, Chung SC. Evaluation of a selective transport medium for gastric biopsy specimens to be cultured for Helicobacter pylori. *J Clin Microbiol* 1998; **36**: 3048-3050 [PMID: 9738066]

24 **Xia HX**, Keane CT, Chen J, Zhang J, Walsh EJ, Moran AP, Hua JS, Megraud F, O'Morain CA. Transportation of Helicobacter pylori cultures by optimal systems. *J Clin Microbiol* 1994; **32**: 3075-3077 [PMID: 7883907]

25 **Grove DI**, McLeay RA, Byron KE, Koutsouridis G. Isolation of Helicobacter pylori after transport from a regional laboratory of gastric biopsy specimens in saline, Portagerm pylori or cultured on chocolate agar. *Pathology* 2001; **33**: 362-364 [PMID: 11523941]

26 **Debongnie JC**, Delmee M, Mainguet P, Beyaert C, Haot J, Legros G. Cytology: a simple, rapid, sensitive method in the diagnosis of Helicobacter pylori. *Am J Gastroenterol* 1992; **87**: 20-23 [PMID: 1728119]

27 **van der Hulst RW**, Verheul SB, Weel JF, Gerrits Y, ten Kate FJ, Dankert J, Tytgat GN. Effect of specimen collection techniques, transport media, and incubation of cultures on the detection rate of Helicobacter pylori. *Eur J Clin Microbiol Infect Dis* 1996; **15**: 211-215 [PMID: 8740855 DOI: 10.1007/BF01591356]

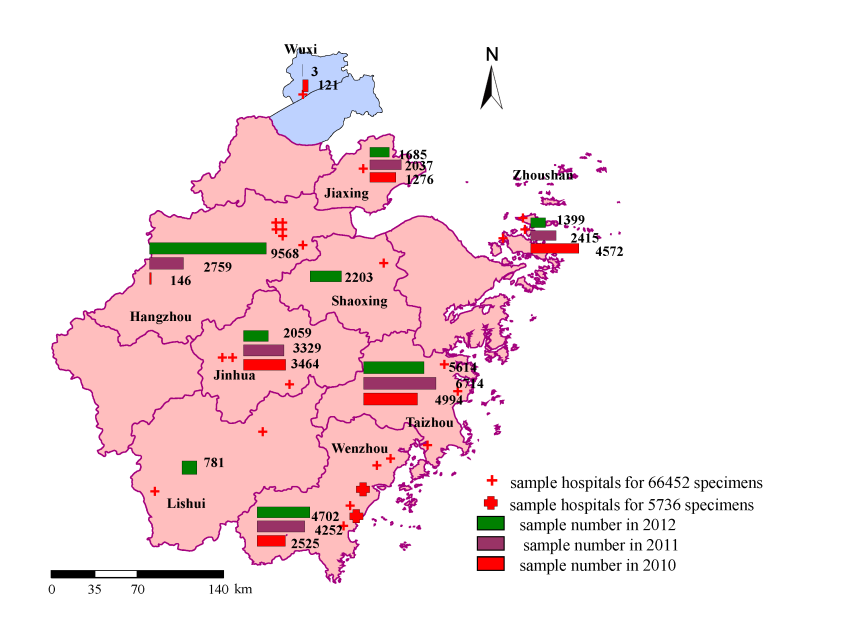
28 **Veenendaal RA**, Lichtendahl-Bernards AT, Peña AS, Endtz HP, van Boven CP, Lamers CB. Effect of transport medium and transportation time on culture of Helicobacter pylori from gastric biopsy specimens. *J Clin Pathol* 1993; **46**: 561-563 [PMID: 8331182 DOI: 10.1136/jcp.46.6.561]

29 **Meunier O**, Walter P, Chamouard P, Piemont Y, Monteil H. [Isolation of Helicobacter pylori: necessity of control of transport conditions]. *Pathol Biol* (Paris) 1997; **45**: 82-85 [PMID: 9097852]

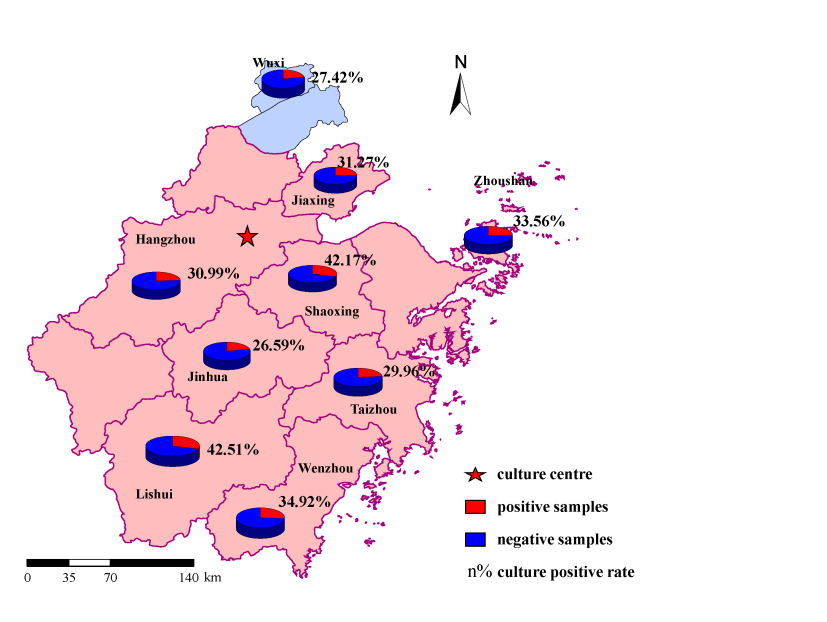
30 **Savarino V**, Zentilin P, Pivari M, Bisso G, Raffaella Mele M, Bilardi C, Borro P, Dulbecco P, Tessieri L, Mansi C, Borgonovo G, De Salvo L, Vigneri S. The impact of antibiotic resistance on the efficacy of three 7-day regimens against Helicobacter pylori. *Aliment Pharmacol Ther* 2000; **14**: 893-900 [PMID: 10886045 DOI: 10.1046/j.1365-2036.2000.00780.x]

**P-Reviewer:** Buzas GM, Yula E **S-Editor:** Qi Y **L-Editor: E-Editor:**

**Figure 1 Samples and the related *Helicobacter pylori* cultural positive rates.** A: Distribution of 26 hospitals and sample numbers from 2010 to 2012 in each city; B: The positive rates in nine cities in Zhejiang and Jiangsu Provinces. The map was drawn by ARCGIS. 9.3. software.

****

**A**

****

B

**Figure 2 Culture positive rates in four different hospitals in Hangzhou.**

**E:\投稿文章\lancet投稿\Figure 2.tif**

**Figure 3 Temperatures and related culture positive rate in six cities within three years.** A: The maximum, minimum and average temperatures in Hangzhou, Jiaxing, Jinhua, Taizhou, Wenzhou and Zhoushan in three years. Tmax: maximum temperature; Tmin: minimum temperature; T: average temperature.B: The culture positive rate curves of six cities in three years.

**E:\投稿文章\lancet投稿\Figure 3.tif**

**Figure 4 Culture positive rates of six cities in Zhejiang Province in January, May, June and July.**

E:\投稿文章\lancet投稿\Figure 4.tif