Antimicrobial susceptibility testing of Helicobacter pylori isolated from patients suffering from gastroduodenal ulcers in a tertiary care hospital

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Abstract
Introduction: The association of Helicobacter pylori with peptic ulcers has gained its importance and has led to ongoing research for the most appropriate treatment. Combinations of multiple drugs presently are having best cure rates.

Materials and Methods: Based on clinical signs and symptoms, 96 patients were selected for study purpose, out of which 34 patients were positive for Helicobacter pylori. Identification was done based on Direct Gram stain, Gram stain of the colony, positive oxidase, catalase and urease test. Antimicrobial susceptibility testing was carried out with agar dilution method using 5-drugs namely clarithromycin, levofloxacin, metronidazole, amoxicillin and tetracycline.

Results: Out of the 34 isolates, 6 isolates were resistant to clarithromycin (17.6%), 5 isolates were resistant to levofloxacin (14.7%), 20 isolates showed resistance to metronidazole (58%), only one isolate showed resistance to amoxicillin (2.9%) and 2-isolates showed resistance to tetracycline (5.8%). In this study higher resistance was seen to metronidazole as the drug is widely used in India for the treatment of amoebic dysentery. Compared to males, female patients showed higher resistance to metronidazole (92%) due to its use in the treatment of trichomonas vaginalis infection.

Conclusion: Most patients are prescribed initial Helicobacter pylori eradication treatment without culture and antimicrobial susceptibility testing. Emergence of resistance is thought to be due to prior antibiotic use hence irrational use of antimicrobial should be stopped to prevent drug resistant.

Keywords: Antimicrobial susceptibility test, Agar dilution method, Helicobacter pylori, Gastroduodenal ulcer.

Introduction
Eradication of Helicobacter pylori is a major advance in the management of gastroduodenal ulcers. Many peptic ulcer patients who were not taking NSAIDs were diagnosed with Helicobacter pylori infection and these ulcers heal faster and are permanently cured by successful eradication therapy. The development of regimens for eradication of Helicobacter pylori has progressed at an impressive rate for example, Omeprazole 20 mg O.D; Clarithromycin 250 mg b.d. and Tinidazole 500 mg b.d. can eradicate over 90% of individuals with Helicobacter pylori infection and decrease the risk of gastric cancer if it is given early enough. There are various combinations of therapies given for Helicobacter pylori infection. The heavy metal bismuth is an ancient antibacterial agent that was widely used in pre-antibiotic era. Bismuth is available in several preparations.1 The preparation most widely used is tri-potassium di-citrate bismuthate (TDB). TDB blocks adhesion of Helicobacter pylori to epithelial cells and accumulates along the bacterial membranes and inhibits its enzyme urease.2 In 1981, Martin et al. found the remission of duodenal ulcer after they had been healed with bismuth.3,4 The main side effects are blackening of the stool, nausea, neurotoxicity which may lead to encephalopathy and impaired renal function which is reversible on stopping the drug.5 Nitroimidazoles act by binding to bacterial DNA and they are more effective than other agents as they are rapidly distributed in all tissues and the salivary concentration of drug is same as in blood.6 They also appear to pass freely in the gastric juice. Drug secreted in saliva and then swallowed may contribute to intragastric concentration of Nitroimidazoles. Another advantage of this drug is that unlike other antibacterials its effect is undiminished at lower pH.7,8 Side effect includes unpleasant taste of the drug as it is secreted in saliva, nausea, diarrhea, rashes, headache, drowsiness, neurotoxicity, leucopenia and some patients suffer from Disulfiram – like effects with Metronidazole, so alcohol should be avoided. Amoxicillin is widely used drug in treatment against Helicobacter pylori but it is much less effective against Helicobacter pylori when the pH is low.9 Tetracycline is ineffective when given as monotherapy. Tetracycline acts by inhibiting bacterial protein synthesis by binding to the bacterial ribosomes. Side effects of Tetracycline include gastrointestinal disturbances, erythema and headache. Clarithromycin exerts a bacteriostatic effect by preventing bacterial ribosomes from producing proteins.8 Macrolides such as Erythromycin are effective against Helicobacter pylori infection as it is secreted in gastric juice but Clarithromycin is more potent than other Macrolides and tissue penetration is also excellent. Acid suppressing drugs will raise the intragastric pH where antibiotics like Doxycline may become ineffective.10,11 Eradication rates with Amoxicillin monotherapy are as low as 2%.12 Resistance to Nitroimidazoles is not an all-or-none phenomenon but may still respond to high doses of this drug. Different mutations like insertion and deletion of transposons, missense and frame shift mutation
involving the vdxA gene have been identified in Metronidazole resistance. As discussed before that the remission of duodenal ulcer is high after treatment with Bismuth hence combination therapies have evolved as better results can be obtained.

**Dual Therapy Regimens**

Eradication rates of 70% have been reported by combining Bismuth with Nitroimidazole, Furazolidone, Amoxicillin, Ciprofloxacin, Oxacillin and Nalidixic acid. Omeprazole with Amoxicillin have been tried with eradication rates ranging from 28% to 92%. Katelaris et al. obtained eradication rate of 72% when Omeprazole was given along with Clarithromycin as the bioavailability of Clarithromycin is increased due to Omeprazole. Eradication rates of 36% to 52% were obtained when Omeprazole was given with other macrolides like Erythromycin, Azithromycin and Roxithromycin. The most effective way to eradicate Helicobacter pylori is still with triple therapy because of higher eradication rates. In 1990, World Congress of Gastroenterology in Sydney recommended triple therapy containing either TDB or bismuth subsalicylate along with Tetracycline and Metronidazole for 2 weeks. Tetracycline can be replaced with Amoxicillin. Eradication rates of 85% were obtained using TDB with Metronidazole and Oxacillin. The most effective way to eradicate Helicobacter pylori is still with triple therapy because of higher eradication rates. In 1990, World Congress of Gastroenterology in Sydney recommended triple therapy containing either TDB or bismuth subsalicylate along with Tetracycline and Metronidazole for 2 weeks. Tetracycline can be replaced with Amoxicillin. Eradication rates of 85% were obtained using TDB with Metronidazole and Oxacillin.

**Triple Therapy Regimen**

Currently more promising approach to eradicate Helicobacter pylori is with an acid suppressor and two antibiotics. The rate of eradication with Amoxicillin alone was 23% but it elevated to 75%, when given along with omeprazole and Metronidazole for 2 weeks and it has also dealt with Metronidazole resistance. An important finding of Bell’s group is that same results were obtained if the regimen was taken for one week instead of two weeks. Bazzoli’s one week regimen containing Omeprazole, Tinidazole and low dose of Clarithromycin increased the eradication rate up to 95%. Lamouliatte et al. obtained an eradication rate of 90% by using Omeprazole, Amoxicillin and Clarithromycin. Amoxicillin seems better than Tetracycline in triple therapy with Omeprazole; while Tetracycline seems better in triple therapy with bismuth based regimens. Quadruple therapies have been described but are now regarded as unnecessary.

**Aims and Objectives**

The prevalence of resistant strains varies widely between different geographical locations and also reflects the local usage of this drug. The aim of this study is to determine the antimicrobial susceptibility of Helicobacter pylori in our region which is necessary for its eradication.

**Materials and Methods**

Based on clinical signs and symptoms, 96 patients were selected for study purpose, out of which 34 patients were positive for Helicobacter pylori. Identification was done based on Direct Gram stain, Gram stain of the colony, positive oxidase, catalase and urease test. Anti microbial susceptibility testing was carried out using Clarithromycin, Levofloxacin, Metronidazole, Amoxicillin and Tetracycline. The antimicrobial susceptibility testing was carried out using agar dilution method as it is approved by CLSI and is a reliable method usually carried out as reference method for evaluation of the accuracy of other testing methods. The disadvantage of this method is that, it is laborious, difficult to perform and time consuming. All the isolates were inoculated into BHI broth and then 2 µL was delivered to Mueller-Hinton Agar plates. The plates were incubated at 37°C under microaerophilic conditions for 72 hours. The MIC value was the lowest antibiotic concentration completely inhibiting the visible growth of the bacteria. The drugs were said to be resistant when the MICs were above the breakpoint for Helicobacter pylori. The MIC for Amoxicillin is 2 µg/ml, Tetracycline is 4 µg/ml, Metronidazole is 8 µg/ml, Levofoxacin is 1 µg/ml and for Clarithromycin it is 1 µg/ml. The final concentrations of the drug ranged from 0.015 to 64 µg/ml.

**Results**

As shown in table-1 the Anti microbial susceptibility testing was carried out on the 34 isolates of Helicobacter pylori of which 6 isolates were resistant to Clarithromycin (17.6%), 5 isolates were resistant to Levofoxacin (14.7%), 20 isolates showed resistance to Metronidazole (58%), only one isolate showed resistance to Amoxicillin (2.9%) and two isolates showed resistance to Tetracycline (5.8%).
Table 1: Resistance Pattern of Various Drugs Used against Helicobacter pylori Infection

| S. No. | Antibiotic     | Resistance to antimicrobials in 34 patients | Percentage (%) |
|--------|----------------|--------------------------------------------|----------------|
| 1.     | Clarithromycin | 6                                          | 17.6           |
| 2.     | Levofloxacin   | 5                                          | 14.7           |
| 3.     | Metronidazole  | 2                                          | 58             |
| 4.     | Amoxicillin    | 1                                          | 2.9            |
| 5.     | Tetracycline   | 2                                          | 5.8            |

Out of the 34 isolates, Helicobacter pylori were isolated from 21 males and 13 females.

Table 2: Resistance pattern to antimicrobials in Males and Females

| Antibiotic     | Resistance to drug in males (21 No.) | Resistance to drug in females (13 No.) |
|----------------|--------------------------------------|----------------------------------------|
| Clarithromycin | 4 (19%)                              | 2 (15.38%)                             |
| Levofloxacin   | 2 (9.5%)                             | 3 (23%)                                |
| Metronidazole  | 8 (38%)                              | 12 (92.3%)                             |
| Amoxicillin    | --                                   | 1 (7.6%)                               |
| Tetracycline   | 2 (9.5%)                             | --                                     |

Fig. 1: Bar Diagram Showing Resistance Pattern of Various Drugs Used against Helicobacter pylori Infection

Fig. 2: Bar Diagram Showing Resistance pattern to antimicrobials in Males and Females
Discussion

The prevalence of resistance to Metronidazole has been reported to range from 8 to 80% in different countries.\(^{25}\) Our study also showed higher resistance to Metronidazole as the drug is widely used in India for the treatment of Amoebic dysentery. Compared to males, female patients showed higher resistance to Metronidazole (92%) due to its use in the treatment of Trichomonas vaginalis infection\(^{26}\) (Table 2). In our studies the resistance to Clarithromycin was seen to be second highest. Clarithromycin resistance possibly results from the use of the antibiotic in the pediatric, pulmonary medicine and otorhinolaryngology fields. Global Clarithromycin resistance rates have increased from 9% in 1998 to 17.6% in 2008.\(^{27}\)

The primary resistance to Fluoroquinolones has been reported to range between 2 and 22% in different countries or regions\(^{25}\) as compared to our study which showed a resistance of 14.7%. The prevalence of resistance to Amoxicillin has fortunately remained low. Nahar et al. has reported resistance to be 6.6%;\(^{28}\) our study showed slightly lower resistance rate of 2.9%. The prevalence of resistance to Tetracycline also has fortunately remained low. It was reported to be less than 2% in most of the studies (Suzuki et al. 2010),\(^{25}\) but our study showed a slightly higher rate of 5.8%.

Conclusion

Most patients are prescribed initial Helicobacter pylori eradication treatment without prior culture and antimicrobial susceptibility testing. Emergence of resistance is thought to be due to prior antibiotic use; hence irrational use of antimicrobials should be stopped to prevent drug resistance.

Our study showed high rate of resistance to Metronidazole and then to Clarithromycin. The reason for high rate of resistance to Metronidazole may be that the drug is widely used in India for the treatment of Amoebic dysentery. Metronidazole resistance was found to be higher in females compared to males due to its wide usage for the treatment of Trichomonas vaginalis infection. Resistance to Clarithromycin would have been due to the extensive use of the drug in pediatrics, otolaryngology and in pulmonary medicine. Resistance to Amoxicillin and Tetracycline was found to be low; hence one of these drugs should be used in dual or triple therapy regimens.

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