



## Original Research Article

Resistance to Amoxicillin and Clarithromycin in *Helicobacter pylori* isolates in a tertiary care hospitalLavanya Jeyamani<sup>1,\*</sup>, Jayalakshmi Jayarajan<sup>2</sup>, Ventatakrishnan Leelakrishnan<sup>3</sup>, Mukundan Swaminathan<sup>3</sup><sup>1</sup>Dept. of Microbiology, SRM Medical College Hospital and Research Center, Tamil Nadu, India<sup>2</sup>Dept. of Microbiology, PSG Institute of Medical Sciences and Research, Tamil Nadu, India<sup>3</sup>Dept. of Gastroenterology, PSG Institute of Medical Sciences and Research, Tamil Nadu, India

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## ABSTRACT

**Introduction:** Amoxicillin and Clarithromycin are the two most often chosen first line drugs in eradication therapy for *H. Pylori*, owing to their better efficacy. Unlike other bacterial infections, therapy here is not based on individual susceptibility report for this bacterium. Rather an empirical regimen is chosen based on the prevalent resistance report in a given population. Current study is aimed at determining the resistance rate in our study population for amoxicillin (AMX) & Clarithromycin (CLA) and to determine if these two drugs can be continued as first line regimen.

**Materials and Methods:** Gastric biopsy samples from 165 dyspeptic patients obtained through endoscopy was cultured in Brucella Chocolate agar (BCA). Among them 46 samples were showing sufficient growth of *H. Pylori* for antibiotic susceptibility testing. MIC for AMX and CLA was determined by E-strip method based on standard guidelines.

**Results:** 91.3 percent of isolates were susceptible or moderately susceptible to CLA. Percentage of isolates within the proposed epidemiological cut off for AMX was 76.

**Conclusion:** *H.pylori* isolates in our study population had resistance rate well below the cut off (20%) for CLA and it could be safely used as first line regimen in treating patients from this region of the country. On the contrary, more than 20% of the strains had MIC above the proposed Epidemiological cut off for AMX. Hence an increase in dosage and frequency of dosage, in combination with a potent anti-*H.pylori* drug could be used in treatment. Clinical trials are required to study efficacy of AMX based regimen in non susceptible patients. Serial monitoring of biological resistance is required for both these drugs, to determine the change in susceptibility pattern at the earliest.

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## 1. Introduction

*Helicobacter pylori* is the causative agent of peptic ulcer and different types of gastritis. It has also been associated with gastric carcinoma in East Asian countries. Infection is treated with a simple combination therapy of two antibiotics and proton pump inhibitors with or without Bismuth sulphate. Empiric drugs are chosen based on the data of the existing susceptibility pattern in a population obtained through different studies and

clinical outcome of the treatment. Though a number of antibiotics (amoxicillin, clarithromycin, metronidazole, levofloxacin, tetracycline, etc) are available, predominantly chosen first line drugs, used worldwide are Amoxicillin (AMX) and Clarithromycin (CLA). These two drugs have a better efficacy owing to their ability to achieve higher concentration in GI mucosa when combined with proton pump inhibitors (PPI) and a far lesser side effects compared to other Anti- *H.pylori* drugs. They tend to achieve clearance of infection in shorter duration. In addition CLA and AMX produce bare minimum insult to the normal GI microbiome. Studies have proved that combination

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of Metronidazole and Tetracycline is known to achieve maximum eradication rate. However the unpleasant side effects associated with the regimen are very high and patients tend to default in this therapy.<sup>1</sup>

*H.pylori* is not an exception in the current trend of escalating antibiotic resistance among various pathogens. There has been increasing reports of antibiotic resistance posing a difficulty in treating patients based on standard empirical regimen. Culture and antibiotic susceptibility testing for every suspected case is ideal in this scenario. There are several studies and meta-analysis demonstrating the better eradication rates associated with 'Tailored therapy' compared to empirical choice. However owing to the difficulty in transport and culture of the specimen and demanding infrastructure needed for Antibiotic susceptibility testing (AST) performance, individual patient to patient testing is not feasible in all settings.<sup>2,3</sup> Hence percentage of drug resistance must be estimated periodically for deciding on the best empirical regimen in a given region at a given point of time. An antibiotic can be safely chosen as an empirical first line drug as long as eighty percent or more of the isolates in a population are still susceptible to it. Hence the current research was aimed at studying the susceptibility pattern of *H.pylori* for two commonly used first line drugs in treatment - AMX and CLA (by E-strip method).

## 2. Materials and Methods

This observational cross section study was conducted during the period from May 2014 to June 2015 in the Department of Microbiology. Gastric biopsy samples were collected from 165 patients with gastro duodenal diseases by Upper GI Endoscopy. Sample size was derived using the prevalence as 88% as quoted by World Gastroenterology organisation with 90% confidence interval.<sup>4</sup> Convenient sampling method was used to include patients in this study. Patients who had received antibiotics within the past 1 month period and anti-secretory drugs within the past 2 weeks prior to endoscopy were excluded.

The biopsy was taken from the site of lesion or gastric antrum and transported to Microbiology Diagnostic laboratory in Brucella broth (Himedia). Specimens were processed within 2-3 hours from the time of collection to maximise the culture isolation. The biopsy tissue was minced to pieces using a sterile no. 22 scalpel blade on a sterile glass slide. The minced tissue was inoculated on a freshly prepared selective medium; Brucella chocolate agar (Himedia) supplemented with vancomycin 1mg/100ml, polymyxin B 250 IU/100ml and amphotericin B 0.5 mg/100ml.<sup>5,6</sup> This antibiotic combination was chosen based on the common contaminants encountered during processing. Culture plates were incubated under microaerophilic atmosphere 10% CO<sub>2</sub>, 5% O<sub>2</sub>, 85% N<sub>2</sub> at 37° C using Anoxomat (Anaerobic) system. The incubation jar was opened on 3<sup>rd</sup>, 5<sup>th</sup>, 7<sup>th</sup> and 10<sup>th</sup> day and checked for

growth. A negative result was recorded when there was no growth after 10 days of incubation. Growth of *Helicobacter pylori* was seen as tiny (0.5-1 mm), moist, convex and watery colonies (translucent) (Figure 1). Of the 165 samples 52 grew *H.pylori* on culture. Identification was confirmed by Gram stain, catalase, oxidase and urease tests.

Single colony was emulsified in a drop of saline on a clean glass slide. The smear was air dried and fixed with a few drops of methanol. On Gram stain, 's' shaped or seagull shaped Gram negative spirals were seen (Figure 2). Catalase and oxidase was performed by standard slide method and dry filter paper method respectively. *H.pylori* is strongly catalase positive and oxidase positive.

When a few colonies were streaked on the slant of Christensen's urease agar, a rapid (few seconds) color change from yellow to pink occurred, demonstrating the presence of urease and indicating the presence of *H.pylori*.

Isolates confirmed as *H.pylori* were further tested for CLA and AMX susceptibility. Isolated organisms were sub-cultured on antibiotic free Brucella chocolate agar. On an average, the organism took 3-5 days to grow on subculture. Six isolates were lost in this process due to contamination and insufficient growth. The growth was emulsified in Brucella broth and matched to Mc Farland standard of four. CLA and AMX susceptibility was performed by E-strip (Biomerieux) method and the plates were incubated in micro-aerophilic atmosphere at 37°C for 72 hours.<sup>7</sup> Reading on the strip at the point of intersection of the growth was taken as MIC of that isolate. (Figure 3) The breakpoints for the interpretation of susceptibility are given in Table 1. Proposed Epidemiological cut off for AMX is  $\leq 0.125 \mu\text{g/ml}$  for wild-type strain. For isolate with a MIC  $> 0.125 \mu\text{g/ml}$ , there are no definitive guidelines to indicate that the treatment will be a success or failure. Quality controls was performed with *Staphylococcus aureus* ATCC 29213.

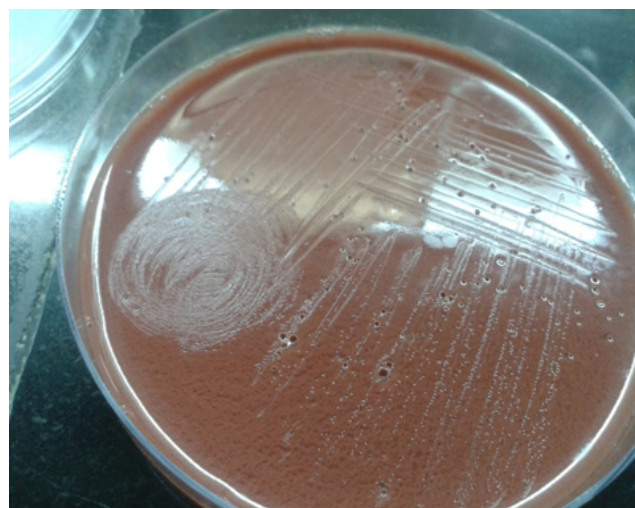


Fig. 1: Growth of *H.pylori* in Brucella chocolate agar

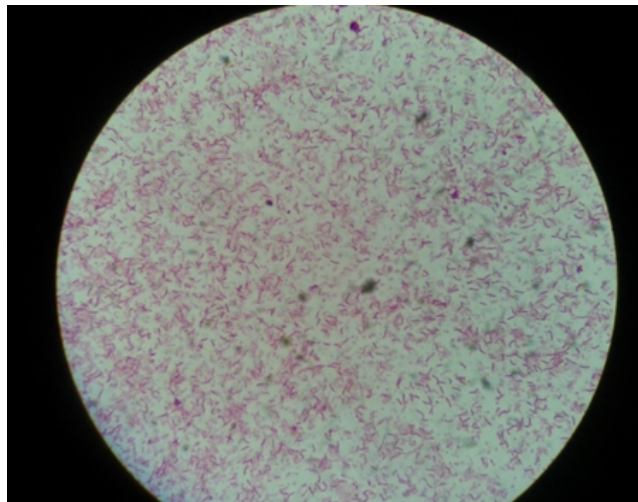


Fig. 2: Gramstain image of *H. pylori* (magnification1000X)



Fig. 3: E-test method of antibiotic susceptibility testing

### 3. Results

Of the 165 patients undergoing biopsy 65% were male and 35% were female. Patient population was distributed between the age group 11-80 years. Out of 165 patients included in this study, 86% (142) had significant endoscopic findings – gastritis (101), gastroduodenitis (11), peptic ulcer (17), etc. Of the 165 patients, 52 (31.5 %) had *H. pylori* infection confirmed by culture.

This bacterium is well known for its fastidious nature. This is a delicate bacterium that fails to grow even with a mild change in environmental conditions. Few isolates were lost during sub-culturing into antibiotic free media for susceptibility testing. Of the 52 isolates 46 survived

subcultures and storage conditions. Table 2 shows the percentage of sensitive and resistance to the drugs tested. Number of isolates in different MIC ranges is shown in Table 3.

### 4. Discussion

Prevalence of *H. pylori* infection was 31.5% in our study population. In endoscopy gastritis followed by gastric ulcer was the most common finding noted among dyspeptic patients. There was an increase in prevalence of infection observed as we moved up in the disease spectrum. *H. pylori* infection was more common in the peptic ulcer cases followed by gastroduodenitis and then gastritis. Among patients with peptic ulcer 76.5% were infected with *H. pylori*, whereas only 28.7% of the gastritis cases had infection. Few patients with no significant lesions on endoscopy were also found to be infected. However the possibility of sub clinical gastric lesion couldn't be ruled out in our study. On studying the antibiotic susceptibility pattern, resistance to CLA was 8.7 percent and susceptible or moderately susceptible was 91.3 percent. Considering the proposed Epidemiological cut off for AMX for Non Wild type as  $>0.125 \mu\text{g/ml}$ , 24% of isolates fell in this category. However clinical studies show that isolates with  $\text{MIC} \leq 0.5 \mu\text{g/ml}$ , were responding to treatment, when patients were treated with AMX 500 mg thrice daily or 1 gm twice daily.<sup>8</sup> Parameters like the steady state concentration ( $2 \mu\text{g/ml}$ ) and half-life of AMX (1.7 hr) are usually taken into consideration before choosing a dosing regimen.<sup>9,10</sup> In the current study, 39 (84.79%) isolates had  $\text{MIC} \leq 0.5 \mu\text{g/ml}$ . Thirty three to thirty five percent of the isolates were having  $\text{MIC} \leq 0.016 \mu\text{g/ml}$  and none of the isolates had  $\text{MIC} \geq 16 \mu\text{g/ml}$  for both the drugs.

Similar figures were documented in most of the Western countries like Spain, Italy, most of the American states and Columbia.<sup>11–16</sup> However studies from different states of India has shown marked variation in CLA and AMX resistance ranging from 3 0-80%, except in Gujarat (4.7%).<sup>17,18</sup> Lower resistance seen in our locale may be due to decreased use of Macrolide drugs as empirical therapy in treating other common infections.

Table 4 shows the possible combinations of antibiogram pattern of the isolates studied. Of the 46 isolates, 38 (82.62%) were either susceptible or moderately susceptible to both the drugs. These patients can be safely treated with AMX and CLA first line regimen. Seven isolates (15.21%) and four isolates (8.69%) were mono-drug resistant when considering different breakpoints for AMX. Nonetheless AMX mono-resistance was more common than the CLA. Resistance to both the drugs was found in 6.5% cases. Triple therapy combination with AMX & CLA couldn't be used in these cases. The prescribed standards for choosing

**Table 1:** MIC( $\mu\text{g}/\text{mL}$ ) cut-off determination of antimicrobial susceptibility

Antibiotics	Susceptible	Intermediate	Resistant
Clarithromycin	$\leq 0.25$	0.5	$\geq 1.0$
Amoxicillin	$\leq 0.125$		$> 0.125$

**Table 2:** Antibiotic susceptibility of *H.pylori* isolates

Antibiotics	No. of isolates (%)		
	Susceptible	Intermediate <sup>a</sup>	Resistant
Clarithromycin	32 (69.6)	10 (21.7)	4 (8.7)
Amoxicillin	35 (76)	-	11 (24)

<sup>a</sup>CLSI guidelines states that the interpretive category intermediate means that the drug can be used to treat infections in sites where the attainable concentration for that drug is higher than the blood/serum concentration. Both AMX and CLA are known to concentrate in GI mucosa and secretions when combined with PPI drugs. Hence the patients with isolates falling within the intermediate zone can still be treated with the respective drugs to attain a clinical cure.

**Table 3:** MIC of *H.pylori* isolates tested against amoxicillin and clarithromycin

MIC Range ( $\mu\text{g}/\text{mL}$ )	No. of isolates within range on testing	
	Clarithromycin	Amoxicillin
$\leq 0.016$	15	16
0.023 - 0.125	12	19
0.19 - 0.25	5	3
0.38-0.75	10	1
1.0-2.0	2	3
4	-	2
8	2	1
12	-	1

**Table 4:** Combined susceptibility results of *H.pylori* isolates

Combined Susceptibility	No. of isolates (considering AMXbreakpoint $\leq 0.125 \mu\text{g}/\text{mL}$ )	No. of isolates (considering AMXbreakpoint $\leq 0.5 \mu\text{g}/\text{mL}$ )
Susceptible to both AMX & CLA	34 (73.91%)	38 (82.62%)
Susceptible to only CLA	6 (13.05%)	3 (6.52%)
Susceptible to only AMX	1 (2.17%)	1 (2.17%)
Resistant/non susceptible to both AMX & CLA	3 (6.52%)	3 (6.52%)
Moderately susceptible to CLA & resistant to AMX	2 (4.35%)	1 (2.17%)

first line drugs in a given population for treating *H.pylori* infection is based on resistance rate of below 2 0% among the population. CLA has a resistance well below the cut off and hence could be safely used as first line drug in treating patients. Given that, AMX break point not been defined accurately and the evidences that the drug could eradicate the infection (If MIC  $\leq 0.5 \mu\text{g}/\text{ml}$ ) when combined with another susceptible drug, we could continue to use it in combinations as a first line drug. However, resistance to AMX was above 20% when recent breakpoints are applied.

## 5. Conclusion

CLA can be used safely as first line drug in treating *H.pylori* in the current study population. In case of AMX, further studies on clinical or microbiological cure

for patients harbouring isolates with MIC  $> 0.125 \mu\text{g}/\text{ml}$  must be performed to decide on whether the patients can be treated with AMX in this scenario. Also susceptibility to AMX must be routinely monitored in future for change in the pattern.

## 6. Acknowledgments

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## 7. Ethical statement

This study was carried out after obtaining Institutional Human Ethical Committee's approval. All procedures performed in this study involving human participants

were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

## 8. Informed consent

Patients were enrolled after obtaining their written informed consent to participate in the study.

## 9. Conflict of interest

All the authors declare that there is no conflict of interest

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