SHORT COMMUNICATION

The prevalence of *Helicobacter pylori* primary resistance towards antibiotics using an Epsilometer-test method

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ABSTRACT

An epsilometer-test method was used to determine MIC values of several antibiotics against 29 *Helicobacter pylori* isolated from gastric antrum of dyspepsia patients. Isolates with resistance towards antibiotics were 6.9% - 65.5% but these were tetracycline-sensitive. Eight isolates showed multi-resistance towards two antimicrobial agents. The high resistance strains towards metronidazole is alarming.

Keywords: *Helicobacter pylori*, Epsilometer test, antibiotic resistance

INTRODUCTION

*Helicobacter pylori*, a ubiquitous bacterium, lives in the human gastroduodenal mucosa. Chronic infection by this bacterium may increase the risk of developing severe gastric diseases such as peptic ulcer and gastric cancer. The prevalence of *H. pylori* infection in Malaysia is comparatively low at 35.9% compared to other Asian countries. Previous epidemiological studies of *H. pylori* in Malaysia consistently showed racial differences in the prevalence of *H. pylori* infection. Indian-Malaysians had the highest prevalence rate, followed by Chinese-Malaysians and the lowest prevalence rate was detected among the Malays (Ang *et al.*, 2005; Sasidharan *et al.*, 2008; Alfizah *et al.*, 2010).

The Maastricht IV Consensus Report has recommended three lines of treatment in managing *H. pylori* infection (Malfertheiner *et al.*, 2012). The treatment regimens consist of triple therapy (1\(^{st}\) line treatment), quadruple therapy (2\(^{nd}\) line treatment) and sequential therapy (3\(^{rd}\) line treatment). A Malaysian population report showed that *H. pylori* were highly resistant against metronidazole (37.4%) but showed low resistance against levofloxacin (1%) and clarithromycin (2.1%), as well as worldwide high resistance for clarithromycin and metronidazole (Ahmad *et al.*, 2011, Ghostasliou *et al.*, 2015).

Primary and acquired resistance of *H. pylori* strain towards antibiotics is believed to be the main factor of treatment failure besides low levels of antibiotic penetration into the gastric mucosa, inactivation of antibiotics caused by the effect of low gastric pH, and patient's treatment compliance (Brouzet *et al.*, 2003; Megraud and Lamouliatte, 2003; Wu *et al.*, 2012). Metronidazole resistance occurs more among women compared to men (Kim *et al.*, 2001). Mono-antibiotic-resistance occurs more among young patients compared to older patients.

Even though the primary resistance of *H. pylori* towards antibiotics is low, a study of the pattern of resistance and its prevalence associated with age, gender and race among the dyspepsia patient population in Malaysia have yet to be reported. Therefore, in this study, the prevalence of *H. pylori* primary resistance towards five antibiotics, amoxicillin, metronidazole, tetracycline, levofloxacin and clarithromycin was determined among dyspepsia patients using the Epsilometer test (E-test).

MATERIALS AND METHODS

Patient information was obtained from the recorded database of 88 dyspepsia patients at UKM Medical Centre, PPUM from years 2005, 2006, and 2012 using a purposive sampling method. *Helicobacter pylori* strains were cultured from the stomach antrum or corpus biopsy.
and only 29 isolates were selected for further study. Patients who had been taking antibiotics within 4 weeks prior to the sample collection were excluded from the sample population.

Isolates were grown onto selective Columbia blood agar (SCBA) supplemented with 7% of sheep blood and incubated under microaerophilic conditions at 37 °C for 5-7 days. *Helicobacter pylori* culture suspension was prepared at 3.0 McFarland turbidity before spreading into a lawn onto Muller Hinton blood agar (MHBA). E-test strips (bioMérieux, USA) were then aseptically placed onto the dried surface of the plates. The minimum inhibitory concentration (MIC) value was defined as the intercept of the elliptical zone of inhibition with the graded antibiotic concentration on the E-test strip as per the manufacturer’s instructions.

The resistance towards only one antibiotic was defined as mono-resistance, while resistance towards more than two antibiotics was defined as multi-resistance. Age range, race and gender-specific prevalence was calculated independently and Chi Square (χ²) of Contingencies statistics were used to compare the association between patient demographic data with prevalence of *H. pylori* primary resistance.

**RESULTS AND DISCUSSION**

The relationship between the primary resistance of *H. pylori* with dyspepsia patients and the demographic data (gender, race and age) is shown in Table 1. There was no significant difference based on the gender of patients with dyspepsia. Results showed that patients of Chinese descent had a higher percentage of dyspepsia compared to the other races. The data also showed that dyspepsia occurred to individuals aged 50 and above. A total of 21 *H. pylori* isolates showed resistance towards all antibiotics, and only 8 isolates were susceptible. Out of this number, the prevalence of *H. pylori* primary resistance among men was found to be higher (13; 44.8%) compared to women (8; 27.5%). The prevalence of primary resistance was highest among isolates from the Chinese-Malaysians (n=8; 27.6%), followed by isolates from the Indian-Malayans (n=7; 24.1%), isolates from other races (n=4; 13.8%) and the lowest was among isolates from the Malays (n=2; 6.9%).

**Table 1:** Relationship between the primary resistance of *H. pylori* among dyspepsia patients and the demographic data (gender, race and age ranges).

<table>
<thead>
<tr>
<th>Descriptive data</th>
<th>Dyspepsia patients (%)</th>
<th>Prevalence Status</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Susceptible (%)</td>
<td>Resistance (%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Total (%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>P-value</td>
</tr>
<tr>
<td><strong>Gender</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Men</td>
<td>15 (51.7%)</td>
<td>6 (6.9%)</td>
</tr>
<tr>
<td>Women</td>
<td>14 (48.3%)</td>
<td>6 (20.7%)</td>
</tr>
<tr>
<td><strong>Race</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Malay</td>
<td>4 (13.8%)</td>
<td>2 (6.9%)</td>
</tr>
<tr>
<td>Indian</td>
<td>8 (27.6%)</td>
<td>1 (3.4%)</td>
</tr>
<tr>
<td>Chinese</td>
<td>13 (44.8%)</td>
<td>5 (17.2%)</td>
</tr>
<tr>
<td>Others</td>
<td>4 (13.8%)</td>
<td>-</td>
</tr>
<tr>
<td><strong>Age range</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(years)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>20-49</td>
<td>9 (31.0%)</td>
<td>2 (6.9%)</td>
</tr>
<tr>
<td>50-79</td>
<td>20 (69.0%)</td>
<td>6 (20.7%)</td>
</tr>
</tbody>
</table>

Different lifestyles between men and women, such as higher smoking rates and alcohol consumption among men might increase the potential in triggering *H. pylori* infection (Paunio et al., 1994). The impact of a multiracial Malaysian population towards the prevalence of primary antibiotics resistance of *H. pylori* was not significant (p=0.240), although early *H. pylori* infection was found to be high among the Malaysian-Chinese population and low among the Malay population. Our findings were in parallel with a report by Sasidharan et al. (2008) where the prevalence of *H. pylori* infection among Malays is considered low at 30.4% and a similar trend was also reported in Singapore (Fock and Ang, 2010). These results showed that *H. pylori* resistance was solely caused by the resistant strain of *H. pylori* and was not influenced by the patient’s ethnic group.

As for the age range factor, dyspepsia patients between 20-49 years old were found to have less antibiotic resistant *H. pylori* strains with only 7 patients (24.1%), while patients between 50 to 79 years old tend to be infected with more resistant *H. pylori* strains, (n=14; 48.2%). Previous studies reported that resistance towards antimicrobial agents on patients was not gender and age-related. Although there were differences in the prevalence of *H. pylori* resistance, we found no significant relationship between percentage of susceptible and resistant *H. pylori* for each demographic data category (gender, race and age range) (p value = p > 0.05 for each category).

The prevalence of resistance recorded using descriptive analysis of resistance of the isolates towards each antibiotic is shown in Figure 1. No resistance was observed for all isolates towards tetracycline while the highest percentage of resistance was towards metronidazole at 65.5%. An effective treatment in managing the infection had significantly reduced the risk of developing gastric cancer in dyspepsia patients without pre-malignant lesions (Wong et al., 2004).

However, resistance towards particular antibiotics used in the treatment is the main cause of treatment failure. Metronidazole and clarithromycin are antibiotics used in first line therapy for the *H. pylori* infection in Malaysia. A study conducted in 2011 by Ahmad et al. showed about 36.4% *H. pylori* strains were resistant towards metronidazole.

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in India (90%), 77.7% in 77 years (2014). However, 2002; Hu et al., 2000). The use of resistance towards either, 2001). Resistance towards metronidazole was due to alteration of strains. Many factors had been attributed to the decreased rates of H. pylori treatment, but most studies were for treatment should be abandoned if clarithromycin resistance is more than 20% (Malfertheiner et al., 2007). In the Malaysian population, clarithromycin is still relevant as the first line therapy in H. pylori infection. However, the increase of clarithromycin-resistance observed in the present study is of great concern and warrants continuous surveillance of antibiotic susceptibility profiles among H. pylori isolates.

Levofloxacin has been used as a rescue antibiotic when there were cases of failure in the first line therapy (Gisbert and Morena, 2006; Saad et al., 2006). Moreover, levofloxacin was also suggested for the second line treatment (Gisbert, 2009). Previous report from other Malaysian hospital showed 6.8% of H. pylori were resistant to levofloxacin (Teh et al., 2014). However, increased trend in levofloxacin resistance were noted in the present study where resistance towards levofloxacin was found in 4 out of 29 isolates (13.8%). In addition, resistance towards amoxicillin was detected in 2 out of 29 isolates (6.9%) in the present study. This is of a great concern as no resistance to amoxicillin among H. pylori strains in Malaysia were reported previously (Ahmad et al., 2011; Teh et al., 2014). Further analysis and molecular detection of amoxicillin resistance is required to confirm the presence of resistance. Amoxicillin is an alternative antibiotic used in the first line treatment of H. pylori. The primary resistance of H. pylori towards amoxicillin was due to low affinity of PBPs (penicillin binding proteins) towards β-lactam antibiotics resulting from the mutation of the pbpA gene in H. pylori. Our findings in Table 2 showed that out of 29 tested H. pylori isolates, 8 (27.6%) showed multi resistance towards at least two types of antimicrobial agents tested with 2 isolates resistant to both metronidazole and amoxicillin (6.9%), 2 isolates were resistant to both metronidazole and levofloxacin (6.9%), 3 isolates were resistant to both metronidazole and clarithromycin (10.3%) and 1 isolate was resistant to metronidazole, clarithromycin and levofloxacin (3.4%). Meanwhile, the remaining isolates were either mono resistant or multi resistant. Out of the total of 13 mono resistant isolates, 11 isolates (37.9%) were found resistant towards metronidazole and 1 isolate each was resistant towards levofloxacin and clarithromycin. Multi resistance also occurs in Mexico, with a prevalence of 30%, in which 18% was resistant towards metronidazole and clarithromycin (Torres et al., 2001). Resistance towards either metronidazole or clarithromycin would cause failure in the H. pylori treatment (Graham, 1998). The high resistance to levofloxacin and clarithromycin advises against their wide empirical use of these antibiotics in eradication regimens (Macias-Garcia et al., 2017).

The Epsilometer test (E-test) has become the gold standard in evaluating H. pylori resistance cases toward antibiotics (Megraud et al., 2001). According McGill et al. (2009) the disc diffusion technique tends to classify less antibiotics resistance, even though a few of the resistant isolates were actually labeled as susceptible. However, these two methods are still suitable for susceptibility testing to determine prevalence resistance towards particular antibiotics. However, the E-test method is more advantageous since it can provide good quantitative

### Table 2: Prevalence of mono or multi resistance.

<table>
<thead>
<tr>
<th>Status</th>
<th>Antibiotics</th>
<th>Frequency</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>No resistance/</td>
<td>Levofloxacin</td>
<td>1 (3.4%)</td>
<td>8 (27.6%)</td>
</tr>
<tr>
<td>Sensitive</td>
<td>Clarithromycin</td>
<td>1 (3.4%)</td>
<td>8 (27.6%)</td>
</tr>
<tr>
<td></td>
<td>Metronidazole</td>
<td>11 (37.9%)</td>
<td>13 (44.8%)</td>
</tr>
<tr>
<td>Mono resistance</td>
<td>Metronidazole + Amoxicillin</td>
<td>2 (6.9%)</td>
<td>2 (6.9%)</td>
</tr>
<tr>
<td></td>
<td>Metronidazole + Levofloxacin</td>
<td>3 (10.3%)</td>
<td>8 (27.6%)</td>
</tr>
<tr>
<td></td>
<td>Metronidazole + Clarithromycin</td>
<td>1 (3.4%)</td>
<td>8 (27.6%)</td>
</tr>
<tr>
<td>Multi resistance</td>
<td>Metronidazole + Clarithromycin + Levofloxacin</td>
<td>1 (3.4%)</td>
<td>8 (27.6%)</td>
</tr>
</tbody>
</table>
results (MIC) useful in determining certain antibiotic doses for treatment.

CONCLUSION

This study can be concluded that the prevalence of \textit{H. pylori} isolated from dyspepsia patients with primary high resistance towards metronidazole. Increasing trend of clarithromycin and levofloxacin resistance among the \textit{H. pylori} isolates in our population is of a great concern and warrant continuous surveillance as both are the key antibiotics in \textit{H. pylori} eradication regimens. All isolates are susceptible to tetracycline and this antibiotic is still reliable to be used as a rescue therapy for \textit{H. pylori} eradication. No significant relationship between prevalence of resistance and demographic data of dyspepsia patients towards primary resistance of \textit{H. pylori} was found in this study. This showed that the development of \textit{H. pylori} resistance does not related to patients’ characteristic. Further study is required to determine the mechanisms of antibiotic resistance among \textit{H. pylori} strains in our population.

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