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Antibiotic Sensitivity to *Helicobacter pylori* Growth

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A B S T R A C T

A combination of different antibiotics was mostly used in H pylori eradication. Eradication of H. pylori likely has a beneficial effect in preventing the development of subsequent gastric dysplasia,a premalignant lesion of gastric Ulcer. This study comprised fifty gastritis peptic ulcers patients, 24 of them were males and 26 were females. These patients were attending the gastroscope clinic at Gastroenterology center (GEC), Mansoura University Hospitals (MUHs), over 6 months, during the period from May 2015 to April 2016, with symptoms and signs suggesting gastritis. H pylori response to both Clarithromycin and Metronidazole antibiotic sensitivity was giving the same figures. In respect to gender, 90.9% of males and 88.2% of females were sensitive to both antibiotics. Tetracycline antibiotic sensitivity was showing that 81.8% of the males and 88.2% of the females were sensitive to Tetracycline antibiotics. H pylori response to both Clarithromycin and Metronidazole antibiotic sensitivity was giving the same figures. In respect to gender, 90.9% of males and 88.2% of females were sensitive to both antibiotics. Tetracycline antibiotic sensitivity was showing that 81.8% of the males and 88.2% of the females were sensitive to Tetracycline antibiotics.

Introduction

Helicobacter pylori Infection

Helicobacter is a genus of Gram negative bacteria possessing a characteristic helical-shape. They were initially considered to be members of the Campylobacter genus, but in 1989 Goodwin *et al.,* published sufficient reasons to justify his new genus name of *Helicobacter*. The *Helicobacter* genus contains about 35 species (Boyanova, 2011).

Some species have been found living in the lining of the upper gastrointestinal tract, as well as the liver of mammals and some birds. The most widely known species of the genus is *H. pylori*, which infects up to 50% of the human population (Yamaoka, 2008). The genus *Helicobacter* belongs to the Epsilon subdivision of the *Proteobacteria*, order *Campylobacterales*, family *Helicobacter-aceae* (Fox, 2002).

H. pylori is a helix-shaped (classified as a curved rod, not spirochaete) Gram-negative bacterium about 3 μm long with a diameter of about 0.5 μm. It is microaerophilic; that is, it contains a hydrogenase which can be used to obtain energy by oxidizing molecular hydrogen (H_2) produced by intestinal bacteria (Olson and Maier, 2002).

It is motile by means of 2-6 sheathed unipolar flagellae; about 3m long with a bulb at the end (O'Toole *et al.,* 2000). Unlike most enteric bacteria, *H. pylori* lacks fimbrial adhesions. Its cell envelope consists of an outer and inner plasma membranes separated by a 30 nm thick periplasm. The dense cytoplasm contains nucleoid material, ribosomes and intracellular polyphosphate granules (Costa *et al.,* 2013).

Helicobacter pylori are recognized as the major cause of gastritis, peptic ulcer, and gastric cancer in humans. The majority of bacteria in the gastric mucosal layer are spiral, but in unfavorable conditions, *H. pylori* can convert to coccoid form. Morphological changes are responses to physical and chemical stresses such as increased oxygen tension, pH changes, extended in vitro incubation, and exposure to antibiotics (Nilsson *et al.*, 2002; Hosseini *et al.,* 2012). *H. pylori* should be cultured as soon as possible after sampling because itis very delicate. Biopsies should be kept in a suitable transport medium for up to 24 h at 4 Ċ. Once isolated, *H. pylori* can be stored frozen at -70 Ċ, in broth with 15:20% glycerol. Many types of medium are available for *H. pylori* culture, including selective agars (e.g., Pylori-agar, Wang media, Skirrow agar, and others), which contain specific antibiotics that inhibit commensal bacteria, and nonselective agars (e.g. Columbia blood agar, blood agar, and others). Cultures are incubated under microaerobic conditions (85% N2, 10% CO_2, 5% O_2) at 35 to 37ºC for at least 7 d before discarding cultures as negative. Morphological characteristics and positive catalase, oxidase, and urease reactions are used for identification of *H. pylori* (Martínez *et al.,* 2016).

H. pylori and hypoacidity/ anacidity

During the early phases of oxyntic gastritis caused by *H. pylori*, gastric acid secretion may be only moderately reduced and *H. pylori* eradication even in patients with some degree of atrophy can augment acid secretion (Tie *et al.,* 2016). In patients with pan-gastritis and oxyntic atrophy, *H. pylori* may not be detectable. It is presumed that *H. pylori* cannot live under these conditions. *H. pylori* may have been replaced by other microorganisms which can live in this situation in the stomach, or alternatively, NH3 production by *H. pylori* urease creates a local milieu too alkaline for the agent itself in a stomach without acid (de Medina *et al.,* 2014). The role of acid for *H. pylori* to thrive is also demonstrated by the effects of acid inhibition in combination with antibiotics in eradication of *H. pylori*, as well as the possible oral spread in the stomach during treatment with inhibitors of gastric acid secretion. Interestingly, the new inhibitor of gastric acid secretion, vonoprazan, belonging to potassium-competitive acid blockers, and probably more efficient in inhibiting acid secretion than proton-pump blockers, seems to be more efficient in combination with antibiotics in eradicating *H. pylori* compared with proton-pump inhibitors (Murakami *et al.,* 2016).

H. pylori infection is dependent on temporal hypoacidity or anacidity for its primary infection, but acidity to survive for a long time. H pylori infection in the antral mucosa causes duodenal ulcers induced by increased gastric acid secretion secondary to slight increased gastrin release from the G cells,

probably due to NH3 production provoked by urease (Helge *et al.,* 2016). When infecting the oxyntic mucosa causing inflammation, the functions (mucous and HCO3− production) of the superficial cells are reduced, predisposing for gastric peptic ulcer. Long-term infection of the oxyntic mucosa causes atrophy and marked reduced gastric acid secretion, leading to gastric hypoacidity and marked hypergastrinemia that probably predisposes for gastric cancer. *H. pylori* do not survive in a too acidic (patients with gastrinoma) or in an anacidic stomach (Scott *et al.,* 1998). The interactions between *H. pylori* and the stomach are very complex, but the pathogenesis of most of the diseases in the stomach and duodenum have somewhat be understood, since *H. pylori* plays a central role in most of these conditions.

Antigenic structure and cell wall

Outer Membrane Proteins

The outer membrane protein (OMP) profile of *H. pylori* strains differs significantly from that of other Gram-negative species as no major OMPs predominate, rather multiple lower-abundance OMPs are observed. Approximately 4% of the *H. pylori* genome encodes an extraordinary large set of OMPs (~64 OMPs) divided into five paralogous gene families and this unusual set of OMPs may be a reflection of the adaptation of *H. pylori* to the unique gastric environment where it is found (Alm *et al.,* 2000).

Lipopolysaccharide (LPS)

LPS is the major component of the bacterial cell wall of Gram-negative bacteria. It is an organic compound found in the outer leaflet of outer membranes which contributes to the structural integrity of the bacteria and protects the membrane. Similar to other Gram-negative bacteria, the LPS of *H. pylori* is essential for the bacteria's survival. The LPS of *H. pylori* consists of an O-specific polysaccharide chain, a core oligosaccharide, and a lipid part called lipid A, embedded in the outer membrane. While LPS is often highly toxic for the host, that of *H. pylori* is low in activation of the host immunological responses (Muotiala *et al.,* 1992). Since they undergo phase variation and antigenic variation within a single strain, this would provide the bacteria with a dynamic adherent phenotype (Edwards *et al.,* 2000).

The main aim of this study includes Calculated and statically analyzed for different antibiotic were used to test *H pylori* response, they were Clarithromycin, Metronidazole, Tetracycline, Amoxicillin and Furazolidone.

Materials and Methods

Subjects

This study comprised fifty gastritis peptic ulcers patients, 24 of them were males and 26 were females. The mean age and standard error were 39.7 ± 2.1 (range: 16- 73). These patients were attending the gastroscope clinic at Gastroenterology center (GEC), Mansoura University Hospitals (MUHs), over 6 months, during the period from May 2015 to April 2016, with symptoms and signs suggesting gastritis.

Gastric biopsy specimens

During endoscopy using Olympus gastroscope (Q20 or Q200, Olympus, Tokyo), six antral biopsy specimens were obtained from adjacent areas of the gastric antrum with Olympus biopsy forceps FB- 24KR (Cap size, 6 mm.). Two specimens directly dipped in 1 ml brain heart infusion broth in a sterile screw capped bottle for microbiological study.

Culture

Two drops of homogenate were inoculated onto agar plate of Dent's medium (Selective medium) and another 2 drops were inoculated onto an agar plate of Chocolate medium (non-selective medium), incubated at 37°C under microaerophilic conditions (Campy pale systems, BBL, Cockeysville, Maryland, USA) for up to 5 days, (Kist., 2006).

Results and Discussion

Antibiotic sensitivity to *H. pylori* growth

Different antibiotic were used to test *H pylori* response, they were Clarithromycin, Metronidazole, Tetracycline, Amoxicillin and Furazolidone.

Cross tabulation between positive gastritis patients to *H. pylori* organism gender and Clarithromycin antibiotic sensitivity with risk estimate between males and females was shown in (Table 1). Cross tabulation between positive gastritis patients to *H. pylori* organism gender and Metronidazole antibiotic sensitivity with risk estimate between males and females was shown in (Table 2). In respect to gender, 90.9% of males were sensitive to both antibiotics and 9.1% of them were resistance to the same antibiotics, 88.24% of females were sensitive with both antibiotics and 11.8% of them were resistant to the same antibiotics. In respect to the antibiotic sensitivity itself of both antibiotics, 40% of the sensitive were males and 60% of them were females. While, 33.3% of the resistant were males and 66.7% of them were females,

Cross tabulation between positive gastritis patients to *H. pylori* organism gender and Tetracycline antibiotic sensitivity with risk estimate between males and females was shown in (Table 3). In respect to gender, 81.8% of the males were sensitive to Tetracycline antibiotics and 18.2% of them were resistant. Where, 88.2% of the females were sensitive to Tetracycline and 11.8% of them were resistant. In respect to the Tetracycline antibiotic sensitivity itself, 50% of the resistant to Tetracycline were males and 50% of them were females. While, 37.5% of the sensitive to Tetracycline were males and 62.5% of them were females,

Cross tabulation between positive gastritis patients to *H. pylori* organism gender and Amoxicillin antibiotic sensitivity with risk estimate between males and females was shown in (Table 4). In respect to gender, 81.8% of the males were sensitive to Amoxicillin antibiotics and 18.2% of them were resistant.

Where, 70.6% of the females were sensitive to Amoxicillin and 29.4% of them were resistant. In respect to the Amoxicillin antibiotic sensitivity itself, 42.9% of the sensitive to Amoxicillin were males and 57.1% of them were females. While, 28.6% of the resistant to Amoxicillin were males and 71.4% of them were females.

Cross tabulation between positive gastritis patients to *H. pylori* organism gender and Furazolidone antibiotic sensitivity with risk estimate between males and females was shown in (Table 5).

In respect to gender, 100% of the males were sensitive to Furazolidone antibiotics and 0.0% of them were resistant. Where, 76.5% of the females were sensitive to Furazolidone and 23.5% of them were resistant, (In respect to the Furazolidone antibiotic sensitivity itself, 0.0% of the resistant to Furazolidone were males and 100% of them were females. While, 45.8% of the sensitive to Furazolidone were males and 54.2% of them were females.

Table.1 Cross tabulation of positive gastritis patients to *H. pylori* organism between Clarithromycin antibiotic sensitivity and Gender with risk estimate between them

			Clarithromycin		P
			Sensitive	Resistance	
Gender	Male	Count	10	1	0.664
		% within Gender	90.9%	9.1%	
		% within Clarithromycin	40.0%	33.3%	
	Female	Count	15	2	
		% within Gender	88.2%	11.8%	
		% within Clarithromycin	60.0%	66.7%	

Table.2 Cross tabulation of positive gastritis patients to *H. pylori* organism between Metronidazole antibiotic sensitivity and Gender with risk estimate between them

			Metronidazole		P
			Sensitive	Resistance	
Gender	Male	Count	10	1	0.664
		% within Gender	90.9%	9.1%	
		% within Metronidazole	40.0%	33.3%	
	Female	Count	15	2	
		% within Gender	88.2%	11.8%	
		% within Metronidazole	60.0%	66.7%	

Table.3 Cross tabulation of positive gastritis patients to *H. pylori* organism between Tetracycline antibiotic sensitivity and Gender with risk estimate between them

			Tetracycline		P
			Sensitive	Resistance	
Gender	Male	Count	9	2	0.518
		% within Gender	81.8%	18.2%	
		% within Tetracycline	37.5%	50.0%	
	Female	Count	15	2	
		% within Gender	88.2%	11.8%	
		% within Tetracycline	62.5%	50.0%	

Table.4 Cross tabulation of positive gastritis patients to *H. pylori* organism between Amoxicillin antibiotic sensitivity and Gender with risk estimate between them

			Amoxicillin		P
			Sensitive	Resistance	
Gender	Male	Count	9	2	0.419
		% within Gender	81.8%	18.2%	
		% within Amoxicillin	42.9%	28.6%	
	Female	Count	12	5	
		% within Gender	70.6%	29.4%	
		% within Amoxicillin	57.1%	71.4%	

Table.5 Cross tabulation of positive gastritis patients to *H. pylori* organism between Furazolidone antibiotic sensitivity and Gender with risk estimate between them

			Furazolidone		P
			Sensitive	Resistance	
Gender	Male	Count	11	0	0.088
		% within Gender	100.0%	0.0%	
		% within Furazolidone	45.8%	0.0%	
	Female	Count	13	4	
		% within Gender	76.5%	23.5%	
		% within Furazolidone	54.2%	100.0%	

Chronic infection caused by *Helicobacter pylori* (*H. pylori*) is associated with chronic gastritis, peptic ulcer disease, and gastric cancer. Eradication of *H. pylori* reduces morbidity of chronic gastritis and incidence of gastric cancer in high-risk population. A combination of different antibiotics was mostly used in H pylori eradication. Chon, *et al.*, (2013) Eradication of *H. pylori* likely has a beneficial effect in preventing the development of subsequent gastric dysplasia, a premalignant lesion of gastric cancer, after endoscopic resection. While dual therapy is comparable to recommended rescue therapies for clarithromycin-resistant *H. pylori* (Gao, *et al.*, 2016), Alboraie *et al.*, (2015), found that bismuth-based quadruple therapy is more effective as a first-line therapy than clarithromycin-based triple therapy for eradicating *H. pylori* in patients with *H. pylori*-related chronic gastritis. As standard triple therapies currently fail to cure 80% of *H. pylori* infections in most populations due to an increased antibiotic resistance, especially to clarithromycin (Tongtawee *et al.*, 2015) therefore the use of clarithromycin-based triple therapy is not advisable as an empiric first-line regimen for *H. pylori* eradication in different world parts. In the present study different antibiotic were used to

test *H pylori* response, they were Clarithromycin, Metronidazole, Tetracycline, Amoxicillin and Furazolidone. The sensitivity of each antibiotic was calculated and statically analyzed.

H pylori response to both Clarithromycin and Metronidazole antibiotic sensitivity was giving the same figures. In respect to gender, 90.9% of males and 88.2% of females were sensitive to both antibiotics. Tetracycline antibiotic sensitivity was showing that 81.8% of the males and 88.2% of the females were sensitive to Tetracycline antibiotics. Amoxicillin antibiotic sensitivity was showing that, 81.8% of the males and 70.6% of the females were sensitive to Amoxicillin antibiotics. Finally Furazolidone antibiotic sensitivity was showing that, 100% of the males and 76.5% of the females were sensitive to Furazolidone antibiotics.

It may be concluded that Hp is dependent on temporal hypoacidity or an acidity for its primary infection, but acidity to survive for a long time. Hp infection in the antral mucosa causes duodenal ulcers induced by increased gastric acid secretion secondary to slight increased gastrin release from the G cells, probably due to NH3 production provoked by urease. When infecting the oxyntic mucosa causing inflammation, the functions (mucous and HCO_3^- production) of the superficial cells are reduced, predisposing for gastric peptic ulcer. Long-term infection of the oxyntic mucosa causes atrophy and marked reduced gastric acid secretion, leading to gastric hypoacidity. HP does not survive in a too acidic (patients with gastrinoma) or in an anacidic stomach. The interactions between Hp and the stomach are very complex, but we now understand the pathogenesis of most of the diseases in the stomach and duodenum, since Hp plays a central role in most of these conditions. A combination of different antibiotics (Clarithromycin, Metronidazole, Tetracycline, Amoxicillin and Furazolidone) was mostly used in H pylori eradication. Eradication of *H. pylori* likely has a beneficial effect in preventing the development of subsequent gastric dysplasia, a premalignant lesion of gastric Ulcer.

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