INCIDENCE OF ANTIBIOTIC RESISTANT STRAINS OF *HELICOBACTER PYLORI*: CAN IT EXPLAIN THE LOW CURE RATE OF THE INFECTION?

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Proposal Summary

The term antibiotic resistance refers to the ability of specific microorganisms, principally bacteria, to withstand the effects of an antibiotic. Over the years, increases in the prevalence of antibiotic resistance have been observed in the United States. Resistance to antibiotics can be caused by a variety of things. The many consequences of antibiotic resistance will be discussed. The objective of this study is to determine if the incidence of antibiotic resistant strains of H. pylori explains the low cure rate of the infection.

1. INTRODUCTION

Antibiotics are chemicals produced by microorganisms that kill other microorganisms. Antibiotics were once called miracle drugs because they revolutionized treatment of disease by curing bacterial infections (Durham, 2005). Humans, livestock, and pets have benefited from these wonder drugs (Durham, 2005). Antibiotics are not like other drugs because they act specifically against the pathogenic bacteria, but not the individual being treated (Guillemot and Courvalin, 2001). There are approximately 50 varieties of penicillin, 70 cephalosporins, 12 tetracyclines, 8 aminoglycosides, one monobactam, 3 carbapenems, 9 macrolides, 2 newer streptogramins, and 3 dihydrofalate reductase inhibitors presently known in use (Harold, 1992). The pharmaceutical industry has been relatively successful in creating new antibiotics over the past three decades, especially in Japan, the United Kingdom, France, and the United States (Harold, 1992). This success has caused the scientific community and society to become complacent about the potential of bacterial resistance (Harold, 1992).

Despite all of these antibiotics, people still die as a result of an infection caused by resistant bacteria (Harold, 1992). Over the years, increases in the prevalence of antibiotic resistance have been observed in the United States (Hellinger, 2000). The term antibiotic resistance refers to the ability of specific microorganisms, principally bacteria, to withstand the effects of an antibiotic (Wainwright, 1990). From 1980 to 1987, penicillin resistance in *Streptococcus pneumoniae* remained unchanged at 5% of all strains (Hellinger, 2000). Since 1990, the prevalence of penicillin resistance in pneumococci has risen each year (Hellinger, 2000). In 1993, 7.9% of all strains of enterococci and 14% of strains recovered from patients in the intensive care units of hospitals were vancomycin resistant (Hellinger, 2000). By 1997, the surveys reporting resistance found 44% pneumococci strains in circulation to have reduced susceptibility to penicillin, while 30% demonstrated high-level penicillin resistance (Hellinger, 2000). In 1998, 23% of enterocci (*Streptococcocus* Faccalis) recovered from patients in the intensive care units of the Nosocomial Infections Surveillance hospitals were vancomycin resistant (Hellinger, 2000).

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Resistance to antibiotics can be caused by a variety of things. It can be caused by chromosomal mutation, inductive expression of a latent chromosomal gene, exchange of genetic material through transformation (exchange of DNA between different individuals), transduction (bacteriophage), conjugation by plasmids (extra chromosomal DNA), or jumping genes (transposons) (Harold, 1992). Jumping genes describes the ability for cells to acquire transmissible pieces of DNA, for example plasmids (Harold, 1992). They can be transferred horizontally by conjugative transposons that spread resistance to other species (Harold, 1992). It has been postulated that *Escherichia coli* transferred the ability to produce β-lactamase enzymes that destroy compounds with β-lactam nucleus like penicillin into *Haemophilus influenzae* by initially infecting *Haemophilus parainfluenzae* (Harold, 1992). Intergenus spread of resistance can occur between Gram-positive species, such as enterococci and staphylococci, and between *Pseudomonas* or *Enterobacteriaceae* or anerobes such as *Bacteroides* (Harold, 1992). Gram-positive species can transfer to Gram-negative species, but the reverse is uncommon (Harold, 1992).

There are many consequences of antibiotic resistance. Some consequences are higher mortality, greater morbidity, longer hospitalizations, and greater expenses (Murray, 1994). The additional cost imposed on the United States health care system is estimated to be between $100 million and $30 billion, annually (Hellinger, 2000).

2. OBJECTIVES AND RATIONALE

The objective of the proposed research is to determine if the incidence of antibiotic resistant strains of *H. pylori* explains the low cure rate of the infection. This data would be useful for developing ways to prevent antibiotic resistance in *H. pylori* or find new drugs to control this microorganism. People with the *H. pylori* infection might be able to get relief if the appropriate drug is prescribed.

3. RESEARCH DESIGN

3.1 Test Subjects

H. pylori infection will be determined by observing the presence of *H. pylori* on histological examination of gastric biopsies and the contemporary detection of a positive rapid urease test obtained from patients suspected of harboring this bacterium. *H. pylori*-infected patients with dyspeptic symptoms will be selected. Dyspeptic symptoms will be defined as at least three months of epigastric pain, upper abdominal discomfort, heartburn, early satiety, nausea and vomiting, and regurgitation. Each symptom will be scored from 0 to 5 regarding frequency and intensity. Subjects will be randomly assigned to one of two treatment options. A computer will generate a list of random numbers and these numbers will be printed. They will be hand delivered to the patients containing one of the therapies. Then, the patients will take the medication as an ordinary prescription.

3.2 Antrum Biopsy Preparations and *In Situ* Tests

(Procedure for biopsies and assessment of resistance will be conducted as outlined by Realdi et. al, 1999). In order to determine if the subjects have *H. pylori*, biopsies will be taken and examined. Four biopsies will be taken from the antrum of each patient. Two biopsies will be removed from the angulus and two will be removed from the corpus of the stomach using sterilized forceps and washed endoscopes. Biopsy specimens will be fixed in 10% buffered formalin. Two biopsies from the angulus, from the antrum, and from the corpus will be stained with hematoxylin-eosin and Giemsa stains and

visualized using light microscopy. The density of *H. pylori* will be calculated. A pathologist, who will be unaware of the clinical data, will assess gastritis using the Sydney system.

Biopsies will be tested for urease activity to confirm that subjects have *H. pylori*. The biopsy from the antrum will be placed into a 6% urea solution and will be tested for urease activity. A positive result will be scored when the solution changes color from yellow to pink after approximately 24h in incubation.

3.3 Isolation of *H. pylori*

In order to verify bacterial growth as *H. pylori*, the biopsies will be cultured. The antral biopsy from all patients will be transported at room temperature in *Portagerm pylori* medium (BioMerieux, S.p.A., Rome, Italy) for culture. To culture *H. pylori*, a tissue sample will be streaked onto Columbia agar plates. Samples will be incubated at 37°C in 12% CO_2 and 100% relative humidity atmosphere. Bacterial growth will be identified as *H. pylori*.

The minimal inhibitory concentration for clarithromycin, amoxicillin, metronidazole, tetracycline, and doxycycline will be determined using the Epsilometer test to identify resistant *H. pylor*i strains. This test is a simple, rapid, reliable method to determine the MIC's of antimicrobial agents. The E-Test makes use of a rectangular plastic strip device that has predefined, continuous exponential gradient of antibiotic concentrations that correspond to MIC dilutions (Mendoza, 1998). This plastic test device has an interpretative scale corresponding to 2-fold MIC dilutions indicated on the surface of the strip (Mendoza, 1998). The strip is applied onto the surface of an inoculated Meuller-Hinton agar plate and the drug on the strip is released and diffuses in the agar (Mendoza, 1998). Plates will incubate for 72h at 37°C. This E-Test will be performed three times to check for validity.

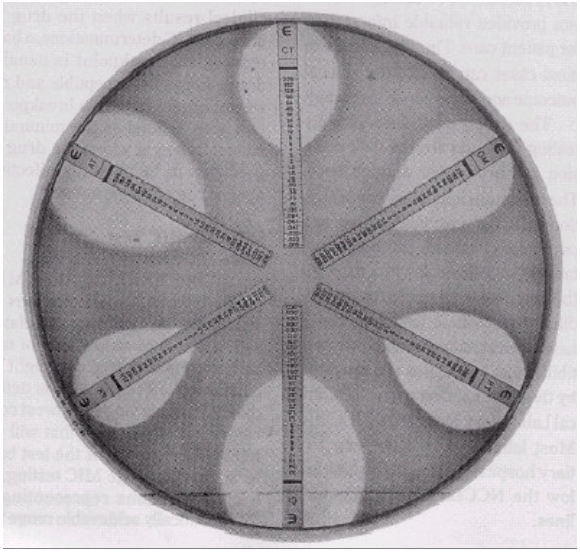

Figure 1: E-Test Plate (Mendoza, 1998)

The following criteria will be used to define resistant *H. pylori* (see Table 1 below).

Table 1: The Criteria for Defining Resistant *H. pylori*

Type of Resistance	Minimal Inhibitory Concentration (MIC) greater than the following value (in µg/ml)
Amoxicillin	8
Clarithromycin	2
Metronidazole	8
Doxycycline	2
Tetracycline	2

It is important that these five antibiotics be chosen because they are the ones that are mostly used in therapies. Cure rates ranging from 80% to 95% usually are expected with triple therapies (Realdi et al., 1999). The therapies consist of bismuth or a proton pump inhibitor and a combination of two antibiotics, usually amoxicillin, clarithromycin, tetracycline, or metronidazole (Realdi et al., 1999). However, the efficacy of anti-*H. pylori* treatment is increasingly being undermined by antibiotic resistance of *H. pylori*, especially to clarithromycin and metronidazole (Realdi et al., 1999).

3.4 Assessment

Treatment success will be the absence of *H. pylori* organisms on histological examination of gastric biopsies obtained from the angulus, antrum, and gastric corpus.

3.5 Compliance and Side Effects (Realdi et al., 1999)

Verbal and written instructions will be given to patients. A phone number will be available for questions or concerns the patients may have. The patients will be asked to count all tablets they take each day. They will need to return the medicine boxes on their next visit. When the patients complete treatment, they will be evaluated for compliance or side effects at a first endoscopy follow-up by a physician. Compliance will be scored according to how many days the medication was taken. It will be good if at least 90% of the pills have been taken. Side effects will be mild (did not limit daily activities), moderate (limited daily activities to some extent), or severe (made daily activities almost impossible).

4. SIGNIFICANCE OF PROPOSED RESEARCH AND IMPACT

In the past, *H. pylori* infection was susceptible to many different antibiotics (Realdi et al., 1999). Presently, the successful treatment of it is challenging (Realdi et al., 1999). The problem of resistant strains of *H. pylori* seems to be consistently increasing (Realdi et al., 1999). This specific problem warrants new approaches, including cost-effective analysis of therapies (Realdi et al., 1999). Thus, if we could determine if the incidence of antibiotic resistant strains of *H. pylori* explains the low cure rate of the infection, then we could develop ways to prevent antibiotic resistance in *H. pylori* or find new drugs to control this microorganism.

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