Cultural characteristics and antibiotic susceptibility pattern of *Helicobacter Pylori* isolated from dyspepsia patients

Bolanle A. Adeniyi,[1] Temitope O. Lawal,[1] Jesse A. Otegbayo,[2] Olayiwola A. Oluwasola,[3] Georgina N. Odaibo,[4] Samuel O. Ola,[2] Clement A. Okolo,[3] Adegboyega Akere,[2] Aderemi O. Kehinde[5]

Departments of [1]Pharmaceutical Microbiology, [2]Medicine, [3]Pathology, [4]Virology and [5]Medical Microbiology, University of Ibadan, Nigeria

Abstract

Helicobacter pylori consist in a helical shaped Gram-negative bacterium, approximately 3 micrometers long with a diameter of approximately 0.5 micrometers. It has 4-6 flagella. It is microaerophilic and tests positive for oxidase, catalase and urease. With its flagella, the bacterium moves through the stomach lumen and drills into the mucus gel layer of the stomach. In humans, *H. pylori* have been associated with peptic ulcers, chronic gastritis, duodenitis and stomach cancer. It is widely believed that in the absence of treatment, *H. pylori* infection, once established in its gastric niche, persists for life. The aim of this research is to study the cultural characteristics and antibiotic susceptibility pattern of *H. pylori* strains isolated from southwest Nigeria. The cultural characteristics and antibiotic susceptibility pattern of *Helicobacter pylori* strains isolated from gastric mucosal antral biopsy specimens collected from 43 of 52 dyspepsia patients in the University College Hospital Ibadan, Oyo State, Nigeria, were determined using standard microbiological methods for *Helicobacter pylori* isolation. The 43 isolates were subjected to 23 different antibiotics and each of the antibiotics demonstrated a variable degree of activity against the isolates. Among the antibiotics to which the organism was most susceptible are: ofloxacin (30 µg) 100% activity, ciprofloxacin (5 µg) 97.67% activity, gentamicin (120 µg) 95.35 activity, amikacin (30 µg), kanamycin (30 µg) and chloramphenicol (30 µg) each 90.70% activity, clarithromycin (15 µg) 93.02, while the less active antibiotics are: augmentin (30 µg) 23.26% active, amoxycillin (25 µg) and metronidazole (50 µg) each 27.91% active and clindamycin (2 mg) 30.23% active. From the result of the antibiotic susceptibility pattern of the strains of the organism, 95.35% of the total isolates are multi drug resistant. Resistance was developed to, among others, augmentin (30 µg), amoxycillin (25 µg), metronidazole (50 µg) and clindamycin (2 mg).

Introduction

Helicobacter pylori consist of a helical shaped Gram-negative bacterium, approximately 3 micrometers long with a diameter of approximately 0.5 micrometers. It has 4-6 flagella. It is microaerophilic and tests positive for oxidase, catalase and urease.[1] *Helicobacter pylori* produce adhesins that help its adhesion to epithelial cells. They produce a large amount of urease enzymes that are localized inside and outside of the bacterium. Urease metabolizes urea (which is normally secreted into the stomach) to carbon dioxide and ammonia (which neutralizes gastric acid). The survival of *H. pylori* in the acidic stomach is dependent on urease, and it would eventually die without it. The ammonia that is produced is toxic to the epithelial cells, and together with the other products of *H. pylori* (including protease, catalase and certain phospholiphase) causes damage to those cells.

Several strains of *H. pylori* are known, and the genomes of two have been completely sequenced (see http:genolist.pasteur.fr/*pylori*Gene).[2] Study of the *H. pylori* genomes is aimed at understanding pathogenesis, the ability of this organism to cause disease. Infection of *H. pylori* may be symptomatic or asymptomatic. It is estimated that up to 70% of infection is asymptomatic and that about two-thirds of the world population are infected by the bacterium, making it the most widespread infection in the world. Actual prevalence of the infection varies from country to country. In humans, *H. pylori* have been associated with peptic ulcers, chronic gastritis, duodenitis and stomach cancer. It is widely believed that in the absence of treatment, *H. pylori* infection, once established in its gastric niche, persists for life.[3,4] *Helicobacter pylori* infection is transmitted orally by means of fecal matter through the ingestion of waste-tainted food or water.

In peptic ulcer patients where infection is detected, the normal treatment procedure is eradicating *H. pylori* to allow the ulcer to heal. The standard first-line therapy is a one week triple therapy amoxicillin, clarithromycin and a proton pump such as omeprazole, pantoprazole or rabeprazole, and the use of metronidazole instead of amoxycillin in those allergic to penicillin.[5,6] Resistance of *H. pylori* to metronidazole and macrolides has emerged worldwide and now constitutes a major problem in therapy.[7-9] This justifies the increasing use of culture in testing for *H. pylori* infection because it is the only diagnostic method that allows the susceptibility of this organism to antimicrobial agents to be assessed. The objective of this research is to study the cultural characteristics and antibiotic susceptibility pattern of *H. pylori* strains isolated from southwest Nigeria.

Correspondence: Bolanle A. Adeniyi, Department of Pharmaceutical Microbiology, University of Ibadan, Nigeria. Tel. +23.4802.3451480.
E-mail: baadeniyi@yahoo.co.uk

Key words: *Helicobacter pylori*, cultural characteristics, antibiotics susceptibility pattern, multi

Contributions: BAA, project planning, susceptibility testing, results evaluation, manuscript writing; TOL, susceptibility testing, results evaluation, manuscript writing; JAO, project planning, patients examinations, endoscopies; SOO, AA, collection of biopsy from patients; AOO, AOK, project planning; GNO, CAO, results evaluation.

Funding: this work was funded by a MacArthur Multidisciplinary Research Grant to the authors.

Conflict of interests: the authors report no conflict of interests.

Received for publication: 24 January 2012.
Revision received: 13 May 2012.
Accepted for publication: 10 July 2012.

Gastroenterology Insights 2012; 4:e21
doi:10.4081/gi.2012.e21

Materials and Methods

Biopsy specimens and culture

Gastric mucosal antral biopsy specimens were collected from each of 52 consecutive dyspeptic patients (35 females aged 10-80 years; 17 males aged 10-90 years) undergoing endoscopy. Gastric biopsy specimens were placed in sterile tubes containing 0.5 mL of transport medium consisting of sterile physiological normal saline, and were kept at 4°C. The interval between the removal of the specimens and the inoculation onto culture media did not exceed 3 h. The biopsy specimens were removed from the transport tubes and placed in fresh sterile tubes containing 0.5 mL saline. The biopsy specimens were then finely mixed in a tissue grinder to dislodge the organisms. One hundred microliters (100 µL) from each

solution were placed for isolation on agar plates. The plates were incubated in 100% humidity at 37ºC for up to seven days in a microaerophilic gas mixture composed of 10% CO_2, 5% O_2, and 85% N_2 (Campy-Pak; Unipak S.p.A, Milan, Italy). The agar plates were checked for growth from day 3 through day 7. An isolate was identified as *H. pylori* on the bases of positive catalase, oxidase, and urease reaction, typical colony morphology (small round colonies), and the presence of characteristic curved gram-negative bacilli on Gram-strained smears. The identified colonies were then sub-cultured in alkaline peptone water.

Susceptibility testing

The isolates were grown in alkaline peptone water to their exponential growth phase and standardized to 0.5 McFarland standards (10^8 CFU/mL); 200 µL of the standardized cell suspensions were evenly spread over the solidified Mueller-Hinton agar plates and the plates were allowed to dry. Concentrations ranging between 5-300 µg/mL (except clindamycin which was in milligrams, mg) of 23 antibiotics in discs (4 mm) were placed on the agar surface using a sterile forceps. The following antibiotics were tested: augmentin, amoxycillin, erythromycin, tetracycline, cloxacillin, gentamicin, cotrimoxazole, chloramphenicol, nitrofurantoin, nalidixic acid, ofloxacin, clarithromycin, metronidazole, cefsulodin, cephradine, vancomycin, tobramycin, kanamycin, clindamycin, amikacin, ciprofloxacin, amoxicillin/clavulanic acid and ampicillin. The plates were incubated under microaerophilic condition as above for 48-72 h after which the zones of inhibition were measured for active drugs.

Results

There were no organisms isolated from samples 1 and 2 hence no further tests were carried out. The Gram negative organisms isolated from samples 14, 17, 20, 31, 41 and 45 gave positive result for catalase and urease tests whereas they tested negative for oxidase test. Isolates from the other (n=44) samples were Gram-negative rods, tested positive for catalase, urease and oxidase tests. An isolate was identified as *H. pylori* on the basis of positive catalase, oxidase, and urease reactions, typical colony morphology (small round colonies), the presence of characteristic curved Gram-negative bacilli on Gram-stained smears and its growth in a microaerophilic environment. The diameter zone of inhibition was interpreted as resistance when the zone size is 6 mm or under, intermediate when the zone size is 7-10 mm and sensitive when the zone size is 11 mm or over.

The percentage susceptibility of *Helicobacter pylori* strains to various antibiotics are shown in Table 1.

Discussion

Culture of *H. pylori* has two major advantages. Firstly, it allows antimicrobial susceptibility testing and secondly, isolates obtained by culture can be characterized in detail. Although the sensitivity of culture in experienced and well-equipped laboratories is greater than 95%, other methods for the diag-

Table 1. Percentage susceptibility of *Helicobacter pylori* strains to various antibiotics.

S/N	Antibiotics	N. susceptible isolates	N. partially susceptible isolates	N. resistant isolates	Percentage susceptible isolates	Percentage partially susceptible isolates	Percentage resistant isolates
1.	Augmentin (30 µg)	4	6	33	9.4	13.9	76.8
2.	Amoxicillin (25 µg)	2	10	31	4.7	23.2	72.1
3.	Erythromycin (5 µg)	11	13	19	25.6	30.2	44.2
4.	Tetracycline (10 µg)	4	16	23	9.4	37.2	53.5
5.	Tetracycline (30 µg)	11	16	16	25.6	37.2	37.2
6.	Cloxacillin (5 µg)	11	11	21	25.6	25.6	48.8
7.	Gentamicin (10 µg)	27	5	11	62.8	11.6	25.6
8.	Gentamicin (12 µg)	30	11	2	69.8	25.6	4.6
9.	Cotrimoxazole (25 µg)	12	4	27	27.9	9.4	62.7
10.	Chloramphenicol (30 µg)	33	6	4	76.7	13.9	9.4
11.	Nitrofuratoin (300 µg)	21	17	5	48.8	39.6	11.6
12.	Nalidixic acid (30 µg)	32	6	5	74.5	13.9	11.6
13.	Ofloxacin (30 µg)	42	1	0	97.6	2.4	0.00
14.	Clarithromycin (15 µg)	27	13	3	62.8	30.3	6.9
15.	Metronidazole (50 µg)	5	7	31	11.6	16.3	72.1
16.	Cefsulodin (30 µg)	19	9	15	44.2	20.9	34.9
17.	Cephradin (30 µg)	13	12	18	30.2	27.9	41.9
18.	Vancomycin (5 µg)	12	7	24	27.9	16.3	55.8
19.	Tobramycin (10 µg)	28	8	7	65.1	18.6	16.3
20.	Kanamycin (30 µg)	28	11	4	65.1	25.6	9.3
21.	Clindamycin (2 mg)	7	6	30	16.3	13.9	69.8
22.	Amikacin (30 µg)	28	10	5	65.1	23.3	11.6
23.	Ciprofloxacin (5 µg)	40	2	1	93.0	4.7	2.3
24.	Amoxicillin/clavulanic acid (30 µg)	11	6	26	25.6	13.9	60.5
25.	Ampicillin (30 µg)	11	17	15	25.6	39.5	34.9

nosis of H. *pylori* infection are simpler, prone to less variability, and faster. Culture of *H. pylori* is very important in the search for efficient antimicrobial combinations that eradicate this bacterium from the stomach. In this study, biochemical tests (positive catalase, oxidase, and urease reactions), typical colony morphology (small round colonies), the presence of characteristic curved Gram-negative bacilli on Gram-stained smears, and growth in a microaerophilic environment, were important parameters in the identification of *Helicobacter pylori* from gastric mucosal antral biopsy. *H. pylori* isolates identified based on the above parameters were studied for their antimicrobial susceptibility. Susceptibility testing of *H. pylori* gave varied susceptibility patterns as seen in Table 1. This reveals the therapeutic challenge in the treatment of *H. pylori* infection in most developing countries.

Antibiotic resistance is a major cause of treatment failure.[10] The prevalence of antimicrobial resistance in *H. pylori* shows regional variation both within and between countries. Alternative antibiotics based on local resistance rates may accelerate eradication rates. Clarithromycin resistance has a greater effect on treatment efficacy than nitroimidazole resistance.[11] The widespread and sometimes indiscriminate use of antibiotics in developing counties has resulted in a higher prevalence of resistance than in developed counties.[3] Clarithromycin resistance rates in the USA have a prevalence of 10-12.5%.[12,13] In Canada, clarithromycin resistance is estimated to be less than 4%.[14] In Europe, there is a marked difference between clarithromycin resistance rate in northern, eastern and southern Europe with resistance rates of 4.2%, 9.3% and 18%, respectively.[15] In this study, 6.9% resistance to clarithromycin (Table 1) was recorded and this falls within the rate reported in Europe. The prevalence of secondary clarithromycin resistance is extremely high at up to 60%.[14] Resistance to metronidazole is much more common than resistance to macrolides. In developed countries, approximately 35% of *H. pylori* strains are resistant to nitroimidazoles whereas in developing countries the resistance rates are higher.[16] As seen in the results (Table 1), 72.1% resistance to metronidazole was recorded thus agreeing with the reports that metronidazole resistance is higher in developing countries.[15] Although metronidazole resistance *in vitro* does not always predict treatment failure, there is poor correlation between different methods of metronidazole resistance detection and this may explain differing resistance rates between regions. Metronidazole is more commonly used in developing countries for the treatment of parasitic infections. In developed countries, it is mainly used for dental and gynecological infections, and in some studies, resistance is commonly found in females.[14] The prevalence of amoxicillin resistance is low (<1%). In areas where penicillin is available without prescription, it may be higher. This explains the high rate of resistance to amoxicillin (72.1%) as found in this study. Tetracycline resistance is estimated to be less than 1% but in our study, 37.2% resistance to tetracycline was recorded. This is due to the indiscriminate use of tetracycline in most developing countries. Fluoroquinolones have been being increasingly prescribed in recent years and this has led to increasing resistance rates. In this present study, the result showed very high primary resistance to antibiotics. *Helicobacter pylori* resistance rate was 6.9% for clarithromycin (15 µg), 72.1% for metronidazole (50 µg), 0% for ofloxicin (30 µg), 4.7% for gentamycin (120 µg), 37.2% for tetracycline (30 µg) and 34.9% for ampicillin (30 µg) (Table 1). Studies have shown that eradication rates of *H. pylori* were not affected by the duration of treatment or the indication for treatment.[17]

Conclusions

In this era of increasing clarithromycin use, the effectiveness and the efficacy of standard triple-therapy regimen for *H. pylori* eradication needs to be reassessed.

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