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Helicobacter Pylori Assay and Urine Bacteriology of Patients with Gastritis

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Abstract

Helicobacter pylori are a non-spore-forming Gram-negative bacterium. The cellular morphology may be curved, spiral, or fusiform, typically 0.5 to 1.0 µm in width and 2.5 to 5.0 µm long. The aim of this study is to determine and compare the prevalence of H. pylori infection and urinary tract infections among gastritis patients. The subjects used in this project work comprised of patients with gastritis. A total number of twenty-five (25) patients with gastritis were recruited for this study. The predominant isolates were Escherichia coli (4), Klebsiella spp (2), Enterobacter spp (2), Staphylococcus aureus (3), Streptococcus spp (3) and Proteus vulgaris (2) with Escherichia coli having the highest prevalence of 25%. The antibiotic susceptibility patterns of the various isolates were read using their zones of inhibitions on the sensitivity culture plates, which shows that Ciprofloxacin, Gentamycin, Streptomycin and Refampicin were the most sensitive antibiotics against the gram-positive bacteria isolates (Streptococcus spp and Staphylococcus aureus) while other drugs were found to be intermediate and resistant. The gram-negative organisms (Enterobacter spp, Escherichia coli, Klebsiella spp and Proteus vulgaris) were more sensitive to Augmentin and Gentamycin, while Ofloxacin, Peflacine, Ciprofloxacin, Septrin and Ampicillin were intermediate while the other drugs were resistant. The noninvasive test-and-treat strategy for H. pylori infection is reasonable for younger patients who have upper gastrointestinal symptoms but not alarm symptoms, like the patient in the vignette. Noninvasive testing can be performed with the use of the urea breath test, fecal antigen test, or serologic test; the serologic test is the least accurate.

Keywords: helicobacter pylori; urine, bacteriology;patients; gastritis

Introduction

Helicobacter pylori are a non-spore-forming Gram-negative bacterium. The cellular morphology may be curved, spiral, or fusiform, typically 0.5 to 1.0 µm in width and 2.5 to 5.0 µm long. The spiral wavelength may vary with the age, growth conditions, and species identity of the cells. In old cultures or those exposed to air, cells may become coccoid (Shirai *et al*., 2000).

The genus *Helicobacter* belongs to the ε subdivision of the *Proteobacteria*, order *Campylobacterales*, family *Helicobacteraceae*. This family also includes the genera *Wolinella*, *Flexispira*, *Sulfurimonas*, *Thiomicrospira*, and *Thiovulum*. To date, the genus *Helicobacter* consists of over 20 recognized species, with many species awaiting formal recognition (Fox, 2002). Members of the genus *Helicobacter* are all microaerophilic organisms and in most cases are catalase and oxidase positive, and many but not all species are also urease positive. *Helicobacter* species can be subdivided into two major lineages, the gastric *Helicobacter* species and the enterohepatic (nongastric) *Helicobacter* species. Both groups demonstrate a high level of organ specificity, such that gastric helicobacters in general are unable to colonize the intestine or liver, and vice versa (Fox, 2002).

Helicobacter pylori (*H. pylori*) infection is accepted as the primary cause of chronic gastritis (Suerbaum and Michetti, 2002). Moreover, severe atrophic gastritis, accompanying intestinal metaplasia caused by persistent *H. pylori* infection, is closely related to the development of gastric cancer (Correa, 1992). Although *H. pylori* was discovered more than 30 years ago by Marshall and Warren (1984), which method should be considered as a gold standard for detection of *H. pylori* infection, especially for epidemiological studies, remains unclear.

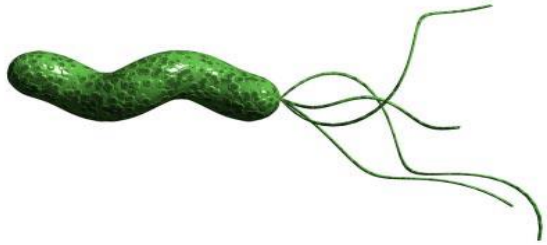

Plate 1: A 10,000x computer-aided design image of *H. pylori* showing curved shape and flagellae that enable the bacteria to propel themselves into the mucus lining of the stomach. (Shirai *et al*., 2000) Currently, several direct diagnostic tests, including histopathology and/or immunohistochemistry (IHC), rapid urease test (RUT), and culture are frequently used as they provide genotype and antibiotic resistance information. However, due to the small amount of bacteria that colonizes the stomach, the direct test sensitivity decreases. Thus, several indirect tests, including antibody-based tests such as serology and urine test, urea breath test (UBT), and stool antigen test (SAT) have been developed to diagnose *H. pylori* infection (Burucoa *et al*., 2013). Among the indirect tests, UBT is one of the most accurate to determine *H. pylori* infection with a sensitivity and specificity of 99% and 98%, respectively (Gisbert and Pajares, 2004). Together with SAT, UBT became the best method to identify active infection, which cannot be detected by serology (Malfertheiner *et al*., 2012).

The public health importance of the discovery of *H. pylori* and its role in stomach diseases was recognized in 2005 by the attribution of the Nobel Prize in Physiology or Medicine to B. Marshall and R. Warren. In the history of Nobel prizes, this is only the third time that the discovery of a bacterium has been acknowledged (Megraud, 2005). For the correct management of peptic ulcer disease and gastric MALT lymphoma, as well as obtaining information on a wide range of diseases associated with *H. pylori* infection, effective diagnostic methods including susceptibility testing are mandatory. Most of the many different techniques involved in diagnosis of *H. pylori* infection are performed in microbiology laboratories.

Urinary tract infection (UTI) is a bacterial infection that affects any part of the urinary tract: kidneys, ureters, bladder and urethra (Stamm and Hooton, 1993; Iyevhobu *et al.,* 2020). Stamm and Hooton, (1993) referred to UTI as a clinical (symptomatic) or subclinical (asymptomatic) disease that may involve just the lower tract or both the lower and upper tracts. Although urine contains a variety of salts and waste products, it usually does not have bacteria in it. But when bacterial gets into the bladder or kidney and multiply in the urine, they cause UTI (Nicolle *et al.,* 1992). Infection may involve only a single site, such as urethra (urethritis), prostrate (prostatitis), bladder (cystitis), kidney (pyelonephritis) but the whole system is always at risk of invasion by bacteria once any part is infected. Urinary tract infections (UTIs) are caused by the presence and growth of micro-organisms anywhere in the urinary tract and are perhaps the commonest bacterial infections of mankind (Morgan and Markenzie, 2001; Adeyeba *et al*., 2002). Urinary tract infection occurs when bacteria is introduced into the urinary system usually through the urethra. When bacteria get into the urinary system they multiply and travel up the urinary tract causing inflammation and irritation along the way (Ayoade *et al.,* 2013; Iyevhobu *et al.,* 2020). It is one of the most common causes of hospitalization and referral to outpatient, having an estimated figure of 150 million per annum worldwide (Hvidberg *et al*., 2000; Stamm and Norrby, 2001; Fakhrossadat *et al*., 2009). Urinary tract infection (UTI) is one of the current infections among teenagers and adults who are sexually active. Screening of asymptomatic subjects for bacteriuria is therefore necessary as bacteriuria has adverse outcomes that can be prevented by antimicrobial therapy (USPSTF, 1996). Apart from that, even the progression of the asymptomatic bacteriuria to the symptomatic UTI in later life can be prevented, which emphasizes the fact that, "prevention is better than cure" (Iyevhobu *et al.,* 2020). Furthermore, untreated asymptomatic bacteriuria can lead to the development of cystitis in approximately 30% of cases, and can lead to the development of pyelonephritis in about 50% of cases. Microbiologically, urinary tract infection exists when significant growth of microorganisms is detected in the urinary tract (Iyevhobu *et al.,* 2020). The infection is generally considered significant and requires treatment when more than 10^5 colony forming units per milliliter (10^5cfu/ml) of urine are present in a properly collected specimen (Brooks *et al.,* 2004).

Infections of the urinary tract are one of the most common infections for which antibiotics are prescribed and are among the most frequently occurring infections arising in the hospital setting (Iyevhobu *et al.,* 2020). Each year UTIs account for more than five to seven million hospital visits, 20 percent of all prescriptions, and require or complicate more than one million hospital admissions in the United States (Schleupner, 1997). UTI affects all age groups, but women are more exposed than men due to the short urethra, absence of prostatic secretion, pregnancy and easy contamination of the urinary tract with faecal flora (Awaness *et al*., 2000). Infection particularly in pregnancy and in elderly can be asymptomatic (Al-Dujiaily, 2000) and is associated with an increased risk of intrauterine growth retardation and low birth weight (Iyevhobu *et al.,* 2020).

Materials and Methods

This study was carried out in Ekpoma, The Headquarter of Esan West Local Government area of Edo State. It is located at latitude 6^o 45^IN and longitude 6^o 08^IE. It is moderately populated with the peoples' occupation being farming and trading (World Gazetter, 2007).

The subjects used in this project work comprised of patients with gastritis. A total number of twenty-five (25) patients with gastritis were recruited for this study.

The research was designed to evaluate the urine bacteriology and *Helicobacter pylori* assay of patients with gastritis. This study was carried out within two (2) months. A record of patients' age and gender was obtained for each subject. Patients with gastritis were recruited for the study. Apparently healthy individuals and patients with no diagnosis of gastritis were excluded for the study.

Sample Collection: Twenty-five (25) Blood and Urine samples was aseptically collected from each patient using sterile, wide-mouthed, leak-proof universal bottles for urine bacteriology and 3 - 5ml of Blood was collected into Ethylinediaminetetraacetic acid (EDTA) bottle for *H. pylori* assay. The age as well as gender of each patient was obtained from the subjects before the collection of samples.

Samples collected from the patients were taken to the Microbiology Laboratory of St Kenny Research Consult, Ekpoma, Edo State for analysis.

***H. pylori* Assay:** Blood samples (3-5 ml) were taken. The blood was kept at room temperature for 1 h; the clot was removed by centrifuging at 1,000-2,000rpm for 2 min in the centrifuge. The resulting supernatant was designated serum. Following centrifugation, the samples were immediately transferred into a clean, sterile universal bottle using a Pasteur pipette. The samples were maintained at 2-8°C while handling. Then the serum was stored at-20°C until use for *H. pylori* assay. Samples that are hemolyzed, icteric or lipemic were excluded from the study. The serum specimens were tested for *H. pylori* using the "HelicotecUT®Plus" test kit.

Microscopic Examination of Urine for Urine bacteriology: A drop of uniformly mixed uncentrifuged urine samples was aseptically placed on a clean grease-free slide and covered with a cover slip**.** It was then examined microscopically to detect the presence of pus cells, epithelial cells, red blood cells, yeast cells, crystal cells and cast cells using 10x and 40x objectives with condenser iris closed sufficiently to give good contrast (Cheesbrough, 2000).

Antibiotics Susceptibility Test: *In vitro* susceptibility tests of the bacterial isolates to antibiotics were done using disc diffusion technique. Zero-point one (0.1) ml of each bacterial isolates prepared directly from an overnight broth culture and adjusted to 0.5 McFarland Standard (NCCLS, 2004) was inoculated using sterile pipette onto each of the nutrient agar media. The commercially available discs containing the following antibiotics: - Penicillin (Pen, 10ug), Ceftazidime, (Caz, 30ug), Streptomycin (Stp, 30ug) Ciprofloxacin (Cpf, 5ug), Gentamycin (Gen, 10ug), Ofloxacin (Ofl, 5ug) Ceftriaxone (Cef, 30ug) and Cotrimoxazole (Cot, 30ug) of oxoid products were aseptically placed on the surfaces of the sensitivity agar plates using a sterile forceps and gently pressed to ensure even contact. The plates were incubated at 37ºC for 24 hours and the zones of inhibition after incubation were observed and the diameters of inhibitory zones were measured in millimeters (mm) using a ruler. The interpretation of the measurement as sensitive and resistant were made according to the manufacturer's standard zone size interpretative manual. The intermediate readings were considered as sensitive for the assessment of the data.

Antibiotic susceptibility test plate showing zones of inhibition[S] and no zone of inhibition[R] around the discs

Results

Microbiological studies were carried out on mid-stream urine and blood samples collected from patients with gastritis in Ekpoma. Twenty-five (25) samples were collected in all which comprise of 12 male and 13 female subjects ranging from age 19 – 33 years.

The predominant isolates were *Escherichia coli* (4), *Klebsiella* spp (2), *Enterobacter* spp (2), *Staphylococcus aureus* (3), *Streptococcus* spp (3) and *Proteus vulgaris* (2) with *Escherichia coli* having the highest prevalence of 25% (Table 1).

Twelve (12) among the twenty-five (25) samples studied were male, while thirteen (13) were female. Twelve (12) out of the twenty-five (25) samples were positive to *H. pylori* assay with subjects within the age range of 26 – 29 years having the highest prevalence of 20% while those within the age range of 19 -21 years have the lowest prevalence of 4% (Table 2).

The prevalence of *H. pylori* according to gender was shown in table 3. Both genders had a prevalence of 6 each but with different percentages due to the difference in number examined.

Table 4 shows the prevalence of microbes isolated from urine samples according to gender. *Enterobacter* spp (1), *Escherichia coli* (1), *Klebsiella* spp (2), *Streptococcus* spp (1) and *Staphylococcus aureus* (1) were isolated from the male subjects and *Klebsiella* spp 2 (12.50) was the predominant isolate from the isolates while *Enterobacter* spp (1), *Escherichia coli* (3), *Proteus vulgaris* (2), *Streptococcus* spp (2) and *Staphylococcus aureus* (2) were isolated from the female subjects with *Escherichia coli* 3(18.75) been the most predominant organism.

More organisms were isolated from subjects within the age range of 26 – 29 years with *Enterobacter* spp (2) and *Escherichia coli* (2) been the highest isolates from this group while those within the age ranges of 19 – 21 years and 20 – 25 years had the lowest no of microorganisms isolated from them (table 5).

The antibiotic susceptibility patterns of the various isolates were read using their zones of inhibitions on the sensitivity culture plates, which shows that Ciprofloxacin, Gentamycin, Streptomycin and Refampicin were the most sensitive antibiotics against the gram-positive bacteria isolates (*Streptococcus* spp and *Staphylococcus aureus*) while other drugs were found to be intermediate and resistant. The gram-negative organisms (*Enterobacter* spp, *Escherichia coli*, *Klebsiella* spp and *Proteus vulgaris*) were more sensitive to Augmentin and Gentamycin, while Ofloxacin, Peflacine, Ciprofloxacin, Septrin and Ampicillin were intermediate while the other drugs were resistant (Table 6).

Isolates were distributed in respect to their various biochemical test reactions as shown (Table 7).

Organisms isolated	No. Isolated	Percentage (%) Prevalence
E. coli	4	25
Klebsiella spp	2	12.5
Proteus vulgaris	2	12.5
Enterobacter spp.	2	12.5
Staphylococcus aureus	3	18.75
Streptococcus spp.	3	18.75
	16	**100**

Table 1: Organisms isolated from Urine samples and percentage prevalence in the study

Age range (years)	No examined	Male	Female	Positive to *H. pylori* assay	Percentage (%) Prevalence
19 – 21	4	1	3	1	4
20 – 25	7	4	3	3	12
26 – 29	7	4	3	5	20
30 – 33	7	3	4	3	12
TOTAL	**25**	**12**	**13**	**12**	**48**

Table 2: Age, Gender and *H. pylori* assay in the study

Gender	No. examined (%)	No. Positive to *H. pylori* assay (%)
Male	12 (48)	6 (50)
Female	13 (52)	6 (46.2)
TOTAL	**25**	**12 (48)**

Table 3: Prevalence of *H. pylori* according to gender

Gender	Organisms isolated	No. Isolated	Percentage (%) Prevalence
Male	*Enterobacter* spp.	1	6.25
	E. coli	1	6.25
	Klebsiella spp	2	12.50
	Streptococcus spp.	1	6.25
	Staphylococcus aureus	1	6.25
Female	*Staphylococcus aureus*	2	12.50
	Streptococcus spp.	2	12.50
	Enterobacter spp.	1	6.25
	Proteus vulgaris	2	12.50
	E. coli	3	18.75
TOTAL		**16**	**100**

Table 4: Prevalence of Microbes isolated from Urine samples according to gender

Age range	Organisms isolated	No. Isolated	Percentage (%) Prevalence

19 – 21	*Staphylococcus aureus* *Proteus vulgaris*	1 1	6.25 6.25
20 – 25	*Streptococcus* spp. *Staphylococcus aureus*	1 1	6.25 6.25
26 – 29	*Enterobacter* spp. *Proteus vulgaris* *E. coli* *Klebsiella* spp	2 1 2 1	12.5 6.25 12.5 6.25
30 – 33	*Staphylococcus aureus* *Streptococcus* spp. *E. coli*	3 1 2	18.75 6.25 12.5
TOTAL		**16**	**100**

Table 5: Prevalence of microorganisms isolated from urine samples in relation to Age in the study

ANTIBIOTICS	***MIRCOORGANISMS***			
Gram Positive Disc	***Staph. aureus***	***Strep.* spp.**		
Ciprofloxacin	S	S		
Norfloxacin	R	S		
Gentamycin	S	S		
Amoxil	R	R		
Streptomycin	S	S		
Refampicin	S	S		
Erythromycin	R	S		
Chloramphanicol	S	R		
Ampiclox	S	R		
Levofloxacin	R	S		
Gram Negative Disc	***E. coli***	***Klebsiella* spp**	***Proteus vulgaris***	***Enterobacter* spp.**
Ofloxacin	R	S	S	S
Peflacine	S	R	S	S
Ciprofloxacin	R	S	S	S
Augmentin	S	S	S	S
Gentamycin	S	S	S	S
Streptomycin	R	S	S	R
Ceporex	R	R	R	R
Naliolixic acid	R	R	R	R
Septrin	S	S	R	S
Ampicillin	R	S	S	S

KEY: S = Sensitive; R = Resistance

Table 6: Distribution of Antibiotic Susceptibility Pattern of Bacterial Isolates

Isolates	Cultural characteristics					Biochemical analysis							Probable Microorganism
	Shape	Elevation	Consistency	Colour	Opacity	Gram	Catalase	Coagulase	Indole	Motility	Oxidase	Urease	
1	Rod	Convex	Mucoid	Cream	Hazy/ Opaque	_	+	_	+	+	_	+	*Escherichia coli*
2	Rod	Raised	Mucoid	Light pink	Opaque	_	+	_	+	_	_	+	*Klebsiella* spp
3	Rod	Flat	Mucoid	Colourless	Hazy/ Opaque	_	+	_	+	+	_	+	*Proteus vulgaris*
4	Rod	Convex	Mucoid	White	Hazy/ Opaque	_	+	_	_	+	_	_	*Enterobacter* spp
5	Cocci in Cluster	Spherical	Moist	Golden brown	Opaque	+	+	+	_	_	_	+	*Staphylococcus aureus*
6	Cocci in chains	Spherical	Moist	Shinning greyish white	Opaque	+	_	_	_	_	_	_	*Streptococcus* spp.

KEY:
+ = Positive
- = Negative
NA = Nutrient agar
MCA = MacConkey agar
A = Acid production
G = Gas

Discussion

Since the discovery of H. pylori and its association with gastritis and peptic ulceration, many tests have been proposed for the detection of this infection. These include tests performed on biopsy samples (urease tests, histological and smear examinations, and culture), noninvasive tests, urea or urea breath tests, and serological tests for specific antibody detection. However, all these tests have limits. Therefore, no test is an ideal test for diagnosis, monitoring of treatment, or epidemiological investigation.

The aim of this work was to ascertain urine bacteriology and helicobacter pylori assay of patients with gastritis. A total of 25 samples (mid-stream urine and blood) were collected in the study period of which 12(48%) were from males and female 13(52%) samples. The prevalence for H. pylori in males and females was 6 (50%) and 6 (46.2%) respectively the prevalence pattern in males and females is shown in table 3.

The prevalence of *Helicobacter pylori* differs both between and within countries, with high rates of infection being associated with low socioeconomic status and high densities of living (Goodman and Cockburn, 2001; Hazel and Francis, 2002). Approximately, 40 and 80% of adult individuals in developed and developing countries are infected respectively. However, the percentage of infected people increases with age, since 50% of infected people were those over the age of 60 compared with around 10% between 18 and 30 years (Brown, 2000). This was not the case in this study, since the highest percentage of patients was among age range of 26 – 29 years. In a large French cross-sectional study, a significantly lower prevalence of *H. pylori* infection was observed in females as compared with males (Broutet *et al*., 2001). However, in this study a highest range of infection was found among females. The infection was associated with variable gastrointestinal illness, chronic gastritis, intestinal metaplasia and ulceration disease. This was in agreement with Pilotto *et al*. (1998) who reported that chronic superficial gastritis associated with *H. pylori* infection is a significant predisposing factor for the development of peptic ulcer, atrophic gastritis, gastric lymphoma and gastric adinocarcinoma.

Many tests are available for diagnosis of *H. pylori* infection. Invasive tests, such as culture, histopathology and biopsy urease test that are required endoscopic biopsy of gastric tissue. Non-invasive tests, such as antibody that was detected in the serum also have been used. Patients with alarming symptoms should undergo endoscopy for the diagnosis of *H. pylori* infection.

H. pylori serologic test is cheap and widely used for the diagnosis of *H. pylori* infection in patients before treatment. Although approved laboratory-based tests have sensitivities and specificities of the commercial *H. pylori*, antibody tests seemed to vary between 60 and 100% (Salomaa *et al*., 2004). The accuracy of serological tests is strongly dependent on the prevalence of *H. pylori* infection. Although it has been recommended that antibody assays be evaluated locally, this has rarely been carried out for different age groups. To avoid any misjudgment in the validation of serological tests for *H. pylori* antibodies in adult subjects, the validation should be carried out separately for different age groups with special emphasis not only on the known *H. pylori* status but also on the presence of atrophic gastritis (Salomaa *et al*., 2004).

Antibiotic resistance has increasingly been recognized as a major cause of treatment failure for *H. pylori* infection. Primary antimicrobial resistance against clarithromycin and metronidazole is now commonplace in several countries (Poon *et al*., 2002). Regional variations in susceptibility and resistance patterns may be ascribed to differences in local antibiotic prescription practices, antibiotic usage in the community and mass eradication programs for *H. pylori* infection as part of gastric cancer prevention strategies. These factors may well be expected to influence success of eradication therapy (Wong *et al*., 2002).

Prevalence of UTIs was more in females 10(62.50%) when compared to males 6(37.50%). This was in agreement with other studies by Bashir *et al* (2008) and Getenet and Wondewosen, (2011). Women are more prone to UTIs than men because, in females, the urethra is much shorter and closer to the anus (Dielubanza and Schaeffer, 2011). This was in consistent with a study by Susan (2005) who concluded that most uncomplicated urinary tract infections occur in women who are sexually active, with far fewer cases occurring in older women, those who are pregnant, and in men. The incidence of UTI increases in males as the age advances because probably because of prostate enlargement and other related problems of old age (Susan, 2005).

Higher proportions of patients were in the age group between 22 - 25 years, 26 - 29 years and 30 - 33 years which had equal numbers followed by those within 19 - 21 years age group. This was in consistent with a study by Getenet and Wondewosen, (2011) in which 53.5% were in the age group between 19-39 years.

E. coli was the most common isolated organism in our study. This was in seen in other studies by Gupta *et al.*, (1999), Moges *et al.*, (2002) and Sibi *et al.*, (2011). The second most common isolated pathogen was *Staphylococcus*

aureus and *Streptococcus* spp in our study accounting for 18.75% each. This was in agreement by Khameneh and Afshar, (2009) and Chin *et al.*, (2011).

In our study *E. coli* was resistant to Ofloxacin, Ciprofloxacin, Streptomycin, Ceporex, Naliolixic acid and Ampicillin. It was sensitive to Peflacine, Augmentin, Gentamycin and Septrin. The similar findings were seen in a study by Bashir *et al.*, (2008) who concluded that the organisms showed resistance to older urinary antimicrobial agents such as ampicillin which indicates that increased consumption of a particular antibiotic can be a pathway to its resistance. Antimicrobial resistance is a natural biological response of microbes to antimicrobial drugs. Resistance may be inherent (Ahmed and Imran, 2008).

Conclusion

In summary, urinary tract infections are very common, especially in women and in healthcare settings. Bacteria, namely *E. coli*, are the main causative agent of these infections. Urinary tract infections are responsible for a large amount of medical expenses and research into vaccination has started due to the problem of antibiotic resistance. Until successful vaccinations are created, prevention is of utmost importance.

The incidence of asymptomatic urinary tract infections appears to be on the increase and there is increase of resistance of bacteria to the commonly used antibiotics. Therefore, there is need to always carry out antibiotic susceptibility assays on isolates before commencing treatment.

The noninvasive test-and-treat strategy for *H. pylori* infection is reasonable for younger patients who have upper gastrointestinal symptoms but not alarm symptoms, like the patient in the vignette. Noninvasive testing can be performed with the use of the urea breath test, fecal antigen test, or serologic test; the serologic test is the least accurate.

Further eradication therapy should not be considered unless persistent *H. pylori* infection is confirmed. Data are lacking to inform the optimal management of recurrent or persistent dyspepsia after noninvasive testing and treatment of *H. pylori* infection. Options include symptomatic acid-inhibitory therapy, endoscopy to check for underlying ulcer or another cause of symptoms, and repeat of the *H. pylori* test-and-treat strategy; other potential reasons for the symptoms should also be reconsidered.

Conflict of interest

The authors declare no conflicts of interest. The authors alone are responsible for the content and the writing of the paper.

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