Original Research Article

Comparing diagnostic performance of Pronto dry rapid urease® and culture to histopathology among endoscopy patients at the Aga Khan University Hospital, Nairobi-Kenya

Abstract

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Aim This study sought to evaluatePronto dry rapid urease® diagnostic test and compare its performance with culture.

Study Design: Cross-sectional study

Place and Duration: From September 2017 to July 2018, across-sectional study was conducted at the Aga Khan University Hospital.

Methodology: Patients attending endoscopyunit at the hospital were randomly sampled to provide gastric biopsy specimen. One specimenwas tested for presence or absence of *H. pylori* using Pronto dry rapid urease® test and another specimen subjected to *in vitro* culture test which were then compared with histology reference results.Test validity and reliability was determined using Graph Pad Prism v5.01.

Results: Of 274study specimens, 121(44%) were positive for histology. Ninety-one (33%) of the study specimen were positive for culture compared to 147(54%) for Pronto dry rapid urease®.Pronto dry rapid urease® test had sensitivity of 100% (97.5%-100%)against 73.6% (64.8%-81.3%) for culture. Specificity was96.1% (91.1%-98.7%) for Pronto dry rapid urease® compared to 35.3% (95% CI 24.1%-47.8%) for culture.Positive predictive value was96.7%(92.5-98.9%)for Pronto dry rapid urease® compared to 97.8% (92.3%-99.7%) for culture. Negative predictive valuewas 100% (97%-100%) for Pronto dry rapid urease® against 82.5% (76.2%-87.7%) for culture. There was significant difference between both Pronto dry rapid urease® and culture test performance with histology in all validity measures, P< 0.001. On the other hand, there was no significant difference between Pronto dry rapid urease® and culture in all validity measures due to overlapping confidence intervals.

Conclusion: Pronto dry rapid urease® out-performed culturein sensitivity and NPV.It would be the method of choice in *H. pylori* detection where histology is untenable and antimicrobial profiling which require culturing the bacterium is needless.

Keywords: *Helicobacter pylori*, Diagnosis, Pronto dry rapid® urease test, Biopsy, Rapid Urease diagnostic tests

Background

Approximately 50% of the world population is known to be infected with *Helicobacter pylori*. *Helicobacter pylori* infection is the major cause of gastric cancer, which accounts for >720000 annual deaths globally(1). It is also the primary causes of other upper gastrointestinal diseases, including dyspepsia, peptic ulcer diseases, heartburn, gastroesophageal reflux disease and even malignant transformation (2). Prevalence of the infection in industrialized countries seems to be decreasing, while in the developing countries it is still high, with figures of up to 90% being reported(3). In Kenya prevalence of *H.pylori*is about 55% in adult and >70% children (4). The burden of the named*H. pylori*-related disease in Kenya is unknown.*Helicobacter pylori*is

transmittedfrom person-to-person through oral–oral and faecal–oral routes(5).Inadequaciesin sanitation practices and wanting sewerage systems common in low social economic populationsare associated with *H. pylori* infection (3).

*Helicobacter pylori*can be diagnosed by non-invasive or invasive methods. The choice of the appropriate diagnostic technique may vary depending on the clinical setting, turnaround time,available laboratory equipment and the presence of specialists such as pathologists.*Helicobacter pylori*diagnostic methods need to be considered individually for their advantages and disadvantages (6). Invasive diagnostic techniques requiring endoscopy are usually preferred in patients with a higher prevalence of gastrointestinal disorders, as well as for their superiority in analyzing the severity of gastritis and detecting premalignant lesions(7). Histopathological determination of gastrointestinal endoscopy is the most commonly performed invasive testand is particularly sensitive for revealing peptic ulcers(8). However, expert pathologists are required for an accurate examination of the samples. It is also intense and requires a well-equipped histopathology laboratory for tissue processing.

Bacterial cultivation is another invasive technique available, though not commonly used in clinical diagnosis of *H. pylori*. Cultivation of *H. pylori*through this method requires specific selective culture agar and specific atmospheric conditions that hinder its routine use in the laboratory as a diagnostic method. Biopsy cultures are the most widely used methods for antimicrobial susceptibility testing, although there is a growing preference for molecular profiling of antimicrobial resistance (9).Rapid urease test is also used to diagnose *H. pylori*on biopsy specimens. It detects urease enzyme production by change of environmental pH, signified by breakdown of urea to ammonia in presence of *H.pylori*.Currently, it's the easiest to perform of all diagnostic methods in Kenya. If valid, it may save patients expenses on repeated visits to the hospital for results. There is evidence of effectiveness of 'test and treat' strategy on *H. pylori* related diseases, and finding a more cost-effective test and one that can give prompt diagnostic results is imperative (10). Pronto dry rapid urease® test is one of the available rapid urease tests. This study sought to evaluate it and compare its performance with culture.

Materials and Method

Study design and area

From September 2017 to July 2018, across-sectional study was conducted at the Aga Khan University Hospitalin Kenya to compare *H.pylori*diagnostic performance of Pronto dry rapid urease® and culture to histopathology. The hospital is one of the major private facilities located in Nairobi.

Sample size and sampling procedure

A total of 274, who were ≥18 old patients with variety of symptoms relating to upper gastrointestinal tract advised by gastroenterologist for endoscopic examination at the endoscopy unit of the hospital were selected by systematic random sampling, where every third patient was enrolled to participate in the study. The study participants represent approximately 8% of all ≥18 years patients seeking these services.Patients taking antibiotics treatment or proton pump inhibitor,expectant mothersand those that had abdominal bleeding days prior to the studywere excluded.

Specimen collection and handling

After an overnight fast, which is staying without eating any form of food or drinking anything for at least 8 hours, upper gastrointestinal endoscopy would be performed with a flexible fiber optic endoscope, where three antral and three corpus specimens were collected in each case. Pronto dry rapid urease® (11) test was performed on the first specimen of the antrum and corpus per manufactures instructions.

Culture was then performed on second specimens within 30 min of collection. This process entailed maceration of biopsies to break up the tissues. Maceration was done by grinding the biopsy tissue in 1.5ml Eppendorf tube containing 0.5ml of Brucella broth with 10% fetal bovine serum using a sterile plastic pestlewhich would then be inoculated on Brucella agar (BD Difco, USA) supplemented with a final concentration of 7% defibrinated horse blood. Inoculation was done, by dipping a flamed inoculation loop that has cooled for some time into the McCartney's bottle containing the homogenized biopsy tissue. The loop was used to streak the surface of an amended Brucella agar blood agar plate. The inoculation loop was flamed until they were red hot after usage. The inoculated plates were then placed inverted into gaspak air-tight container and Campy-gen kit EZ (BD) that generates microaerophilic conditions (80 % N_2, 10% CO_2, and 5%O_2) for the growth of *H. pylori* was placed in the container. The plates were incubated in humidity at 37℃ for up to 7 days. The agar plates were checked for growth from day 3 through day 7. An isolate was identified as *H. pylori* on the basis of positive catalase, oxidase, and ureaseactions, typical colony morphology (small, round colonies), and the presence of characteristic curved gram-negative bacilli on Gram-stained smears.

The third sets of biopsies were subjected to histological examination. Processed tissues were stained with hematoxylin and eosin (H&E staining). Each stained section was examined by two pathologists. A third tie- breaker pathologist's results would be reference in case of discordance in *H.pylori*detection.

Data analysis

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Data was entered into Microsoft Excel ™ 2010 (Microsoft, Seattle, WA, USA). Validity measures which include sensitivity, specificity, Positive Predictive Value (PPV) and Negative Predictive Value (NPV) of both Pronto dry rapid urease® and culture using histology as the reference were calculated by Graph Pad Prism version 5.01at 95% confidence level. Chi square was used to test for significance.

Ethical review

Informed consent was obtained from all participants. This study was approved by the Kenyatta National Hospital/university of Nairobi Ethics Research Committee (Number P241/0 4/2015) and Aga Khan University Hospital research office. No personal identifiers were obtained from participants.

Results

Of 274 patients specimen collected 156 (57%) were male while 118(43%) female, who yielded of 274 study specimens. Of these, 122(44%) were positive for histology. Five (3%) histology negative specimens were positive for Pronto dry rapid urease® test, while 2 (1%) histology negative specimens were positive for culture. There was significant difference in sensitivity and

NPV between Pronto dry rapid urease® test and culture. However, there was no significant difference in specificity and PPV between Pronto dry rapidurease® test and culture due to overlapping confidence intervals. The expert readers disagreed on 2 (<1%) tissue slides requiring a third tie-breaker.

Performance measures for both culture and Pronto dry rapid urease® test are summarized in table 1.

Table 1 Comparison of Performance of Rapid Urease test and Culture against Histology

Test	Sensitivity (95% CI)	Specificity(95% CI)	PPV(95% CI)	NPV(95% CI)
RUT	100% (97.5%-100%)	96.1%(91.1% -98.7%)	96.7% (92.5% - 98.9%)	100%(97% -100%)
Culture	73.6%(64.8%-81.3%)	98.7(95.4%-99.8%)	97.8%(92.3%-99.7%)	82.5%(76.2%-87.7%)

There was no false positive for Pronto dry rapid urease® compared to 2(1.3%) for culture. Likewise false negative results for Pronto dry rapid urease® were 5(4%) compared to 32 (26.5%) for culture. There was significant difference between Pronto dry rapid urease® and the reference histology, P< 0.001. Likewise, culture test performance was significantly different from the reference histology, P< 0.001 Table 2.

Table 2 Comparison of significance between Pronto Dry® Rapid urease testand Culture against Histology

Test	Result	Histology		P-value
		Positive (%)	Negative(%)	
RUT	Positive	122(96.0%)	5(4%)	<0.001
	Negative	0(0.0%)	147(100%)	
Culture	Positive	151(98.7%)	32(26.5%)	<0.001
	Negative	2(1.3%)	89(73.6%)	

Discussion

This study found out that Pronto dry rapid urease® out performedculture in sensitivity and NPV measures. This findings are in agreement with similar studies performed in elsewhere (12, 13,14) Moreover, sensitivity of Pronto dry rapid urease® according to the manufacturer is similar with our findings (15). The observed high sensitivity makes Pronto dry rapid urease® appropriate for screening ahead of the more time consuming confirmatory tests.Furthermore, WHO recommends 'test and treat' strategy 16)and this would come in handy due to its reasonable cost. Likewise the observed high NPV implies that persons presenting at the endoscopy unit of the hospital for*H.pylori* testing who actually do not have the infection are likely to be appropriately classified as such by Pronto dry rapid urease® test. Pronto dry rapid urease® detects urease enzyme production by *H. pylori*. However interpretation of result can be complicated by other urease producing bacteria are present in gastric mucosaRecent treatment with antibiotics, bismuth containing compounds and proton pump inhibitors decreases density of bacteria producing urease enzyme activity, can reduce sensitivity of the Pronto dry rapid urease® (17).

Culture performance on sensitivity and NPV was comparatively lower, which may be due to the fastidious nature of the bacterium and strict microaerophilic environment requirement(10). Only live bacterium can be propagated using culture. The bacterium viability is also known to be easily lost during transportation ahead of culture. For this study we tried to mitigate this by using FBSfor transport and promptly processing specimens for culture. Use of antibioticsby patients can also influence the outcome of culture results as it decreases the density of bacteria from the gastric mucosa.It is possible some participants may have taken antibiotics and could not tell they were those in question.This culture findings are however not unique to this study, since even a study conducted by (18)had similar results. Culture is imperative for antimicrobial susceptibility profiling.

Histology as used in this study would detect both dead and live bacilli in the gastric mucosa. This might explain the positive cases obtained by culture and Pronto dry rapid urease® which were negative with histology. Histology is dependent on interpersonal ability of the pathologist to morphologically identify the bacterium, which might limit the extent to which the method can be used as a 'gold standard'. However, histology has been used in method validation in a number of studies. Histology has the advantage of able to identify *H.pylori*and provide more information on the degree of inflammation and associated pathology (19).

Limitation of this study is that three biopsies would be obtained for each test; culture, histology and Pronto dry rapid urease®. The density and distribution of bacilli in the gastric mucosa can be uneven (20)and can result in over estimation or under estimation of the performance measure.The study should have included patients from all age groups for a more conclusive analysis of results. In addition, a more sensitive test like PCR should have been used to confirm the five histology negative specimens that were positive for Pronto dry rapid urease® test and the two histology negative specimens that were positive for culture.

Conclusions

The performance of Pronto dry rapid urease® was commendable. It outperformed culture in a number of performance measures, and would be useful in *H. pylori* detection especially where histology is untenable and antimicrobial profiling which require culturing the bacterium is needless.

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