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ISOLATION AND IDENTIFICATION OF *HELICOBACTER PYLORI* FROM CULTIVATED AND COMMERCIALLY SOLD VEGETABLES AND SALAD FROM SOME SELECTED TOWNS (NNEWI, AND ODEKPE) IN ANAMBRA STATE

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ABSTRACT

Helicobacter pylori plays an important role in peptic ulcer disease and gastric carcinoma.This study was conducted to isolate and identify Helicobacter pylori from 64 cultivated and 60 commercially sold vegetables and salad materials in Odekpe and Nnewi towns in Anambra state Nigeria using conventional culture technique on selective Columbia blood agar base and antibiotic supplement(Oxoid, England). Identification was done using biochemical tests. Antibiotic susceptibility was also done with Kirby Bauer disc-diffusion method using Mueller Hinton agar (HiMedia Laboratories, Mumbai, India) according to the Clinical Laboratory Standards Institute.(CLSI 2012).Results revealed that a total of 6(9.4%) and2(3.3%) was isolated from vegetables from Odekpe and Salad materials from Nnewi towns with no significant difference ($P>0.05$;$P=0.171$,$X^2=1.873$). Pumpkin leaves was more contaminated in Odekpe 1(16.7%) and leek in Nnewi.2(100%).Unwashed samples recorded higher prevalence than washed, with no significant difference. ($P>0.05$;$p=0.144$,$x= 2.138$). Animals walking around farm, type of animal and source of water for irrigation showed marked significant association with the presence of organism. ($P<0.05$; $P=0.007$, 0.009, 0.002: $X^2=7.30$, 6.84, 15.28).Highest resistance to antibiotics was recorded in Nalidixic acid 8(100%) and Cephalothin 8(100%) and susceptibility to Chloramphenicol 8(100%) and Ciprofloxacin 8(100%). In conclusion, vegetables from Odekpe town were highly contaminated with H. pylori. Unwashed vegetables pose a threat to H. pylori contamination. Knowledge on type of animal walking around the farm, water source used for irrigation should be disseminated as a risk factor to H. pylori spread.

KEYWORDS: *Helicobacter pylori*, Vegetables, Salad, Nnewi, Odekpe.

INTRODUCTION

Helicobacter pylori previously named *Campylobacter pylori*, is a Gram-negative, microaerophilic bacterium found in the stomach, and may be present in other parts of the body, such as the eye (Cotticelli *et al.*, 2006). Helicobacter pylori is recognized as the major cause of gastritis and peptic ulcer and gastric mucosa-associated lymphoid tissue (MALT) gastric lymphoma (Atherton, 2006). It is also linked to the development of duodenal ulcers and stomach_cancer. However, over 80% of individuals infected with the bacterium are asymptomatic and it may play an important role in the natural stomach ecology (Blaser, 2006).

Vegetables and salads are rich and comparatively cheaper source of vitamins. Their high values for minerals and vitamins are undeniable and, in a day, millions of people use the vegetables and salads in their main diet. Therefore, hygienic quality of vegetables and salad has a high importance in public health but sometimes it will be changed and several infections and illnesses will occur. Consumption of these food sources provides taste, palatability, increases appetite and provides fiber for digestion and prevents constipation. Vegetables are raised as complete foods. Vegetables are in contact with soil, polluted water, animal manure and even stool. Therefore, they can easily become contaminated. A previous study showed that soil, water, animal manure and stool (Sasaki *et al., 1999)* are the

main sources of *Helicobacter pylori*. Several studies have confirmed the high presence of *H. pylori* in pasteurized and sterilized food products (Vale *et al., 2010*). Therefore, emphasis on hygiene can be an exceptional way for reducing the load of *H. pylori* in foods. Food products that have been analyzed thus far mainly include milk, meat and vegetables. Among these, milk products are the most studied while vegetables are rare (Vale *et al., 2010*). The association of the infection with consumption of raw vegetables is an additional indirect evidence for the presence of *H. pylori* in water used for irrigation of these vegetables (Quaglia *et al., 2009*). A previous study indicated that poor quality water could represent an important vehicle for *H. pylori* transmission (Mazari-Hiriart *et al., 2008)* In addition to water used for irrigation of vegetables, animal manure used for reinforcement of soil is an additional indirect evidence for the presence of *H. pylori* in vegetables. This bacterium has been isolated previously from cow's fecal samples (safaei *et al., 2011*). Feces of animal and especially cows have been used for reinforced agricultural soil. Fujimura et al. showed that the prevalence of *H. pylori* was 50% in cow feces and 38% in soil samples. Also, this bacteria has been isolated from various animal sources (Tabatabaei, 2012). Contact with cow feces is one of the main sources of vegetable contamination. Foods with water activity higher than 0.97 and pH ranging from 4.9 to 6.0 theoretically provide conditions for the survival of *H. pylori*. Also, the general lack of efficient sanitation in removing or killing pathogens on raw fruits and vegetables may contribute to harbor pathogens (Beuchat, 2002). *H. pylori* is unlikely to grow on most food products, but it is able to survive in a low acid and high moisture environment for extended periods of time, especially if refrigerated. As far as we know, vegetables grown in high moisture soil can allow *H. pylori* development for a long duration of time. An epidemiological association between water sources and the prevalence of H. pylori infection has been identified (Goodman *et al.*1996,) Further evidence for water as a vehicle for transmission has been provided by culture of H. pylori from the feces of infected individual(Kelly *et al*., 1994), maintenance of viability in water, amplfication of H. pylori-specific nucleic acid sequences in water (Hulten *et al.*, 1998) and detection of actively respiring H. pylori in surface and groundwater.

The infection is more common in crowded living conditions with poor sanitation. In countries with poor sanitation, approximately 90% of the adult population can be infected. Infected individuals usually carry the infection indefinitely (for life) unless they are treated with medications to eradicate the bacterium. One out of every six patients with *H. pylori* infection may develop ulcers of the duodenum or stomach. It is also observed that H. pylori can also cause natural infection in other primates (Goodman *et al.*, 1996). To avoid the acidic environment of the interior of the stomach (lumen), *H. pylori* uses its flagella to burrow into the mucus lining the stomach to reach the epithelial cells underneath, where the pH is more neutral. (Amieva, 2008). It adheres to the epithelial cells by producing adhesins, which bind to lipids and carbohydrates in the epithelial cell membrane. In addition to using chemotaxis to avoid areas of low pH, *H. pylori* also neutralizes the acid in its environment by producing large amounts of urease, which breaks down the urea present in the stomach to carbon dioxide and ammonia. The ammonia, which is basic, then neutralizes stomach acid.

MATERIALS AND METHOD

Study area
This research was carried out in the selected towns (Nnewi, and odekpe) in Anambra state

Research design
This is a cross sectional studies designed to isolate and identify *H.pylori* from vegetables and salad

Study population
The research consists of a total of 250 selected vegetable from registered stalls and selected vegetable farm lands in the study area of interest.

For Nnewi town in Nnewi north Local Government Area:100 salad materials(leek-10samples,spring onion -10s,parsley -15s,irish potato-15s,cabbage-10s,lettuce-10,cucumber—10,carrot-10, Green pepper-10).

Odekpe in Ogbaru Local Government Area: 150 vegetable samples (pumpkin leaves-50, scent leave-50,water leaves-50).

Sample size
A total of 250 samples were used using the Daniel mathematical formula (1999).

$N=Z^2\times P\ (1-P)/d^2$
N=Minimum sample size
P=Prevalennce rate of *Helicobacter pylori*=0.648(64.8%) (Walsh and Fass,1997; Ifeanyi *et al.*,*2013*)
D=Desired level of significance=0.05(5%)
Z=Confidential interval=1.96(95% confidence interval).
$N= (1.96)^2\times 0.648(1-0.648)/(0.05)^2$
N=250.5

Sample technique
Simple random sampling technique was used.

Ethical approval
Ethical approval was obtained from the ethical committee Faculty of Health Science and Technology, Nnamdi Azikiwe university, Nnewi Campus and the Departmentment of Medical Laboratory Science.

Issuance of questionnaires
Questionnaires was given to farmers and traders after educating them about the bacterium, it's transmission and infection. Oral interviews was also used as well as

vernacular where necessary. Variable datas like site, type of manure used, was noted.

Collection of Vegetables and salad materials
Different vegetable leaves were randomly collected from farms at different sites,also salad leaves were bought from different markets from different traders.

Sample processing and analysis
Samples were analysed using methods described below:

a. The vegetables were weighed 20gram each for both washed and unwashed sample.
b. The sample for' washed' was washed with clean water in sterile blender (Eleganzer japan).
c. The sample for both washed and unwashed was homogenized with strerile Phosphate buffered saline (PBS).
d. The two samples were pre-enriched with *H.pylori* antibiotic supplement (Oxoid, England) and PBS at Ph of 6.8 and incubated at 37^0C for 7 days at micro-aerophilic temperature.
e. Culture of the pre-enriched sample was done in *H.pylori* Selective Agar (HPSA) (OXOID, England) and selective supplement. Using the convetional cultural method and incubated at micro-aerophilic temperature.
f. Culture of the control strain of *H. pylori* (ATCC 43504) was also done and incubated for 7 days at the same temperature and condition.

Sample identification

1. Gram staining technique
2. Biochemical tests
3. Sensitivity testing
4. Production of hydrogen sulphide
5. Sensitivity to nalidixic acid.
6. Sensitivity to cephalothin

All according to Cheesebrough (2010).

Gram stainig technique
The gram staining reaction was used to to help identify the pathogen as a gram negative flagellated organism.

Method for Gram staining

i. A smear was made on a clean grease free slide and allowed to air dry.
ii. The air dried smear was fixed by heat by passing the slide,smear uppermost through Bunsen flame for three times
iii. It was Covered with crystal violet stain for 60 seconds.
iv. The stain was rapidly washed off with clean water
v. The slid was tipped off all the water and covered with lugols iodine for 60 seconds.
vi. The iodine was washed off with clean water.
vii. It was decolourized rapidly with acetone-alcohol and washed immediately with clean water.
viii. The smear was covered with neutral red for 2min and washed off with clean water.
ix. The back of the slide was cleaned and placed in a draining rack for it to air-dry.
x. Examination of the smear microscopically was first made with×40 objective to check the staining and the distribution of materials,and then with oil immersion objective to report the bacteria and cells.
xi. Biochemical tests

1. Catalase test
The test was used to establish the catalase activitiy of *H. pylori.*

Method

I. 2-3ml of the hydrogen peroxide solution was poured in a test tube.
II. A sterile wooden stick was used to remove some colonies of the test organism asnd immersed in the hydrogen peroxide solution. Care was taken not to take the agar alongside the organism because the agar was supplemented with sheep serum.
III. Bubbles were checked for immediately.

1. Oxidase
Method using an oxidase reagent strip

I. The strip was moistened with a drop of sterile water.
II. Using a piece of stick,a colony of the test organism was rubbed on the strip.
III. Red purple colour was checked for within 20 seconds.

Urease test
Method using Christensens(modifid) urea broth

I. The test organism was inoculated in a bijou bottle containing 3ml sterile Christensens modified urea broth.
II. It was incubated at 35 to 37^oC for 24 hours.
III. A pink colour was checked for in the medium.

Antimicrobial susceptibility testing
Antimicrobial susceptibility testing was performed by the Kirby-Bauer disc diffusion method using Mueller-Hinton agar (HiMedia Laboratories, Mumbai, India) supplemented with 5% defibrinated sheep blood and 7% fetal calf serum, according to the Clinical Laboratory Standards Institute.(CLSI 2012).

Statistical Analysis
The data obtained was organized and subjected to appropriate analysis using statistical package for the social sciences (SPSS).version 20, t test and anova was also used.

RESULT

Table 1: The distribution of *H. pylori* isolated from washed and unwashed vegetables and salad materials cultivated and sold in odekpe town. Pumpkin leave has the highest number of isolates 3(50%).followed by gardenegg leave 2(33.3%) and spinach 1(16.7).

Table 2: The distribution of *H. pylori* isolated from washed and unwashed vegetables and salad materials cultivated and sold in Nnewi town.only leek onion was contaminated with *H,pylori* with a frequency of 2(33.3%).

Table 3: The grand total of frequency of *H. pylori* isolated from washed and unwashed samples from vegetables and salad materials in Odekpe and Nnewi town.

Table 1: The distribution of *H. pylori* isolated from washed and unwashed vegetables and salad materials cultivated and sold in odekpe town.

Name of vegetable	No of Samples	Frequency	Prevalence (%)	Total H. pylori (%)
Pumpkin leave				
Washed	6	1	16.7	3(50)
Unwashed	6	2	33.3	
Scent leaves				
Washed	5	0	0	0(0)
Unwashed	5	0	0	
Water leave				
Washed	5	0	0	0(0)
Unwashed	5	0	0	
Table 1 Contd				
Bitter leaves				0(0)
Washed	5	0	0	
Unwashed	5	0	0	
Gardenegg leave				
Washed	6	1	16.7	2(33.3)
Unwashed	6	1	16.7	
Spinach				
Washed	5	0	0	1(16.7)
Unwashed	5	1	16.7	
Total	64	6	100	6(100)

Table 2: The distribution of *h. Pylori* isolated from washed and unwashed vegetables and salad materials sold in nnewi town.

Spring onion				
Washed	4	0	0	0(0)
Unwashed	4	0	0	
Irish potato				
Washed	3	0	0	0(0)
Unwashed	3	0	0	
Cucumber				
Washed	3	0	0	0(0)
Unwashed	3	0	0	
Leek onion				
Washed	6	0	33.3	2(33.3)
Unwashed	6	2		
		Table 2 Contd		
Cabbage				
Washed	4	0	0	0(0)
Unwashed	4	0	0	
Green beans	5			
Washed		0	0	0(0)
Unwashed	5	0	0	
Carrot				0(0)
Washed	5	0	0	
Unwashed	5	0	0	
Total	60	2	33.3	2(33.3)
Grand Total	124	8	100%	8(100)

Table 3: The grand total of frequency of *h. Pylori* isolated from washed and unwashed samples from vegetables and salad materials cultivated and sold in odekpe and nnewi town.

Name of vegetable	Vegetable leaves isolate from Odekpe and Nnewi		*H. pylori* (%)
	Washed	Unwashed	Total
	ODEKPE(VEGE)		
Pumpkin	1	2	3(50)
Garden egg	1	1	2(33.3)
Spinach	0	1	1(16.7)
	NNEWI(SALAD)		
Leek onion	0	2	2(100)
Total Isolates	2	6	8(100)

TABLE 4: The prevalence and susceptibility pattern of *Helicobacter species* isolated from vegetables and salad cultivated and sold in Odekpe and Nnewi towns in Anambra State. *H.pylori* showed marked resistance of 100% to Cephalothin and Nalidixic acid but 87.5% to Augmentin. There was 100% susceptibility of *H.pylori* to Ciprofloxacine and Chloramphenicol,87.5% to Levofloxacine and ofloxacine,75% to Ampicilin and Amoxicilin,62.5% to Gentamycin,Streptomycin and Erythromycine.

TABLE 5: The risk factors of occurance of *H. pylori* on vegetables and salad sold and cultivated in Odekpe and Nnewi towns in Anambra state. This risk factors and their different frequencies include "washed and unwashed" samples- 62(50%) and 60(50%) respectively. Cultivation by self- "yes" 52(41.9%) and "No" 72(58.1%) respectively. Animals walking around the farm "yes" 29(23.4%) and "No" 95(76.6%) respectively. The type of animal include goat 30(24.2) and none 74(59.7%).The water used for irrigation and its frequency include river 26(21.0%), pond 12(9.7%), stream 12(9.7%), and none 74(59.7%). Treatment of water before irrigation gave 124(100%)"NO" as an answer. washing before display-yes 26 (21.0%), "No"-98 (79.0%). The type of water used for irrigation include river 11(8.9%), pond 1(0.8%), tap13(10.5%), stream 2(1.6%), and none 97(78.2%).The manure used for fertilization include artificial fertilizer23(18.5%),goat feaces 15 (12.1%) and none86(69.4%).care given to vegetable-"spread on cement floor"90(72.6%),cover with tapolene34(27.4%).Use of any bacteriocidal-"yes0(0.0%) "no"124(100%).

TABLE 6: The chi square test of the association of occurance of *H.pylori* on vegetables and salad materials cultivated and sold in Odekpe and Nnewi town in Anambra state with some associated risk factors. There was a significant difference between " self cultivation", "animal walking around the farm", "the type of animal" "the type of water used for irrigation" and the occurance of *H.pylori* on vegetables and salad materials cultivated and sold in Odekpe and Nnewi town in Anambra state with a P value of 0.05,0.007,0.009,and 0.002 respectively.($P<0.05$) There was no significant difference between "material", "washing status", "washing before display" "type of water for washing" "type of manure", the "care given to the vegetables" and the occurance of *H.pylori* on vegetables and salad materials cultivated and sold in Odekpe and Nnewi town in Anambra state with a P value of 0.171,0.144,0.132,0.666,0.075,and 0.328 respectively ($P>0.05$).

Table 4: The prevalence and susceptibility pattern of *Helicobacter species* isolated from vegetables and salad cultivated and sold in Odekpe and Nnewi towns in Anambra State.

Variables	Class	Frequency (%)
Incidence of *Helicobacter spp.*	Present	117(94.4)
	Absent	7(5.6)
Incidence of *Helicobacter pylori*	Present	8(6.5)
	Absent	116(93)
Susceptibility of *Helicobacter pylori* to CHLORAMFENICOL(10mcg)	Susceptible	8(100)
	Resistant	0 (0)
Susceptibility of *Helicobacter pylori* to CIPROFLOXACINE (10mcg)	Susceptible	8(100)
	Resistant	0(0)
Susceptibility of *Helicobacter pylori* to LEVOFLOXACIN *(*5mcg)	Susceptible	7 (87.5)
	Resistant	1(12.5)
TABLE 4 CONTD		
Susceptibility of *Helicobacter Pylori* to GENTAMYCIN(10mcg)	Susceptible	5 (62.5)
	Resistant	3(37.5)
Susceptibility of *Helicobacter pylori.* To AMPICILIN (30mcg)	Susceptible	6(75)
	Resistant	2(25)
Susceptibility of *Helicobacter pylori*	Susceptible	6(75)

to AMOXICILIN(30 mcg)	Resistant	2(25)
Susceptibility of *Helicobacter pylori* to STREPTOMYCIN (5mcg)	Susceptible	5(62.5)
	Resistant	3 (37.5)
Susceptibility of *Helicobacter pylori* to ERYTHROMYCIN(10mcg)	Susceptible	5(62.5)
	Resistant	3 (37.5)
TABLE 4 CONTD		
Susceptibility of *Helicobacter pylori* to NALIDIXIC ACID (10mcg)	Susceptible	0(0)
	Resistant	8(100)
Susceptibility of *Helicobacter pylori* to AUGMENTIN (30mcg)	Susceptible	1 (12.5)
	Resistant	7(87.5)
Susceptibility of *Helicobacter pylori* to OFLOXACIN(5mcg)	Susceptible	7 (87.5)
	Resistant	1(12.5)
Susceptibility of *Helicobacter pylori* to CEPHALOTHIN (30mcg)	Susceptible	0(0)
	Resistant	8(100)

Table 5: The risk factors of occurance of *h pylori* on vegetables and salad sold and cultivated in odekpe and nnewi towns in anambra state.

Risk Factors	Categories	Frequency (Percentage)
Washing of matrials	Washed	62(50.0)
	Unwashed	60(50.0)
Do you cultivate it yourself?	Yes	52(41.9)
	No	72(58.1)
Does animal walk around the farm?	Yes	29(23.4)
	No	95(76.6)
What kind of animal?	Goat	30(24.2)
	None	94(75.8)
What kind of water do you use for irrigation or is around the farm?	River	26(21.0)
	Pond	12(9.7)
	Stream	12(9.7)
	None	74(59.7)
Do you treat water before irrigation?	Yes	0(0)
	No	124(100)
Do you wash vegetable before display in the market?	Yes	26(21.00)
	No	98(79.0)
What kind of water do you wash with?	River	11(8.9)
	Pond	1(0.8)
	Tap	13(10.5)
	Stream	2(1.6)
	None	97(78.2)
What type of manure do you use for cultivations?	Artificial fertilizer	23(18.5)
	Goat feaces	15(12.1)
	None	86(69.4)
How do you care for the Vegetables?	spread on cement floor	90(72.6)
	Cover with tapolene	34(27.4)
Do you use any bacteriocidal agent?	yes	0(0.0)
	No	124(100)

Table 6: The chi square test of the association of occurance of *h.pylori* on vegetables and salad materials cultivated and sold in odekpe and nnewi town in anambra state with some associated risk factors.

Variable	Class	Occurance of *H.pylori*		X^2	P
		Present	Absent		
Material	Salad	2(3.3)	58(96.4)	1.873	0.171
	Vegetable	6(9.4)	58(90.6)		
Washing status	washed	2(3.2)	60(96.8)	2.138	0.144
	Unwashed	6(9.7)	56(90.3)		
Do you cultivate it yourself?	Yes	6(115.5)	46(88.5)	3.830	0.05
	No	2(2.8)	70(97.2)		
Does animal walk around the farm?	Yes	5(17.2)	24(82.8)	7.302	0.007
	No	3(3.2)	92(96.8)		
TABLE 6CONTD What kind of anima walk around?	Goat	5(16.7)	25(83.3)	6.842	0.009
	None	3(3.2)	91(96.8)		
What kind of water do you use for Irrigation?	River	6(23.1)	20(76.9)	15.286	0.002
	Pond	0(0.0)	12(100)		
	Stream	0(0.0)	12(100)		
	None	2(2.7)	72(97.3)		
Do you treat water Before irrigation?	No	8(6.5)	116(93.5)	Constant	
Do you wash before display?	Yes	0(0.0)	26(100)	2.269	0.132
	No	8(8.2)	90(91.8)		
What type of water do you wash with?	River	0(0.0)	11(100)	2.380	0.666
	Pond	0(0.0)	1(100)		
	Tap	0(0.0)	13(100)		
	Stream	0(0.0)	2(100)		
	None	8(8.2)	89(91.8)		
TABLE 6CONTD What kind of Manure do you use?	Artififial fertizer	1(4.3)	22(95.7)	5.193	0.075
	Goat feaces	3(20.0)	12(80.0)		
	None	4(4.7)	82(95.3)		
What care do you give to the Vegetable	Spread on Cement Floor	7(7.8)	83(92.2)	0.956	0.328
	Cover with Tapolene	1(2.9)	33(97.1)		
Use of any bacteriocidal agent	No	8.0(6.5)	115(93.5)	Constant	

DISSCUSSION

In table 1, a total of 8(6.4%) was obtained from odekpe and Nnewi towns, frequency of 1(16.7%) and 2(33.3%) was obtained from washed and unwashed pumpkin leave samples, 1(16.7%) and 1(16.7 %) from washed and unwashed garden egg leaves and finally 1(16.7 %) from unwashed spinach leave from Odekpe town. 2(33.3%) was also isolated from leek onion from Nnewi town. This low prevalence of *H. pylori* could be as a result of difficulty in culturing the organism from the environment. *H. pylori* forms a viable but non culturable form outside the body (Kusters *et al., 1997).* Different studies have proved that diverse method for isolation increases their chances of isolation.some researchers have added mucin (Slomiany *et al., 1987*), ferrosulphate and Sodium pyruvate (George *et al.*,1978, Stern *et al.,1988*).

In Table 6, Higher incidence was obtained from vegetables 6(9.4%) than in salad materials 2(3.3%), probably because vegetables are leafy with broader surface area than salad materials enhancing more chances of contamination from air, droplets infections,thereby habouring contaminats than salad materials. There was no significant difference in the occurrence of H. pylori between the two classes (vegetables and salad) of materials sampled. (P ═0.171; P>0.05).

There was also a high frequency 6(9.7%) of *H. pylori* from unwashed vegetables and salad than in washed 2(3.2%) vegetables and salad, this may be because unwashed vegetables are known to be covered with dirt and sand which are potential harbourers of diversified microbial load, while the microbial load of the washed samples has been reduced by the action of water. Also garden soils contain a lot of humus of which could come from animal or humans sources where most of the *H pylori* are found (Sasaki *et al., 1999*). Most cases especially in Nigeria, the manure used are not bioremediated giving more chances to contamination by pathogens (Beuchat, 2002). There was no significant difference (P═0.144; P<0.05) between the occurrence of *H pylori* in the washing status of samples.

A higher occurrence of 6(115.5%) of *H pylori* was obtained in samples of those who cultivated it

themselves than those who did not, with a significant difference of 0.05 (P=0.05).

In individuals who cultivate it themselves, there tends to be competition among farmers for a healthier plant making them add a variety of other compost to enhance fertility. Most are illiterates and do not know the importance of bioremediation or health effects of using untreated dungs. The healthier and lusher the crop appear, the better chances of easy marketing irrespective of source of compost. To this individual, it is a do or die affair. Most of them also dispose their degradable house -hold waste. Some even use their farms as a source of toileting, increasing the potential hazards involved (Tabatabaei, 2012).

The positive isolates obtained from those who do not cultivate it themselves could be because the traders purchased it from farmers who had already done some form of cleaning on the vegetables prior to selling in the market, thereby reducing the microbial load.

A higher incidence of occurrence of *H. pylori* was obtained in the farms where animals walk around than in those where animals do not walk around with a frequency of 5(17.2%) and 3(3.2%) respectively. A lot of research has proved the association between *H. pylori* and animals (wild and domestic)(Quaglia et al., 2008). These animals serve as natural reservoirs, shedding the organism as they graze on the farm and adding manure to the soil.

Nevertheless, *H pylori* was also isolated from vegetables cultivated in farms where animals do not walk around. This could be because the organism is also found in the soil as natural reservoirs (Sasaki *et al.,1999*).

There was more occurrence of isolates from farms where Goat walk around than in farms where Goat do not walk around with a frequency of 5(16.7%) and 3(3.2%) respectively. A higher occurrence of 6(23.1%) was found with individuals who used river more than those who used ponds and stream. River has been proven to contain multiple diverse microorganisms as it receives tributaries from streams, ponds and effluent from homes, Naturally, it will be less hygienic than ponds and streams (Bartram *et al*., 2010, Bellamy *et al*., 2006), especially in one of the areas sampled(Odekpe in Ogbaru LGA of Anambra state), where the river source is River Niger. Odekpe is still a town under siege by flooding. Despite two years flooding experience, the town has not yet organized their drainage system making the river a high risk contamination source. There is a high significant difference between the different sources of water used for irrigation (p- 0.002; P<0.05).

All the farmers and traders do not treat their water before irrigation. It is well known that *Helicobacter pylori* is present in non-treated water. There is a constant association in this variable for both vegetables and salad materials.

All those who do not wash their salad and vegetables before display had positive isolates of 8(8.2%). Naturally, organisms are reduced from surfaces when they are washed. There was no significant difference between washing and not washing before display (P═0.132; P<0.05).

More *H pylori* isolates was obtained from vegetables of those who use goat faces that in those who used artificial manure. A number of works has associated *H pylori* with animals,especially ruminants (Quaglia *et al*., 2008). There was no significant difference in the kind of manure used. (P═0.075 P<0.05).

More positive isolates was obtained from vegetables of those who spread them on cement floors and other formites. Works has proven that exposure of food materials to air and dust is an easy mode of transmission and source of contamination by diverse microbes (Mahomed *et al.,2007).* Most times these Salad materials are bought by restaurant entrepreneurs and in a lot of cases are in a hurry to satisfy the high demands by their customers, thereby being careless in their hygine. Cement floors are prone to feet of animals and humans as well as air as there is no form of chemical or physical irradiation. Sources of contamination as a result of covering with tapolene could be from anywhere. There is no significant difference between the care given to the vegetables and contamination with *H.pylori*. (P= 0.28; P<0.05).

None of the farmers and traders used bactericidal agent for storage, as a result, they all had *H.pylori* isolates 8(6.5%) in both categories (vegetables and salads). Bacteriocidal agent is known to reduce microbial load as stated by (Ball *et al., 2002).*

There was a marked (100%) resistance of isolates to cephalothin and Nalidixic acid in this study which concurred also in a study by Goodwin *et al (1989*). It is interesting to note that these two drugs are basis of biochemical test specific for this organism (Al-sulami *et al.*,2010). *H.pylori* is resistant to both antibiotics. This is followed by Augmentin (87.5%).It should be noted that soil has natural antibiotics and manure used could come from animals droppings and carcass under antibiotic treatment. Also, 8(100%) susceptibility was seen with Ciprofloxacin and Chloramphenicol, followed by Levofloxacine (87.5%), Ofloxacine (87.5). Ampicilin(75%), Amoxicilin (75%), Gentamycin(62.5%), Streptomycin(62.5%) and Erythromycin (62.5%). This is similar to a study in Iran on which shows the prevalence of resistance of Erythromycin and amoxicillin to be 26.0% and 28.6% respectively(Mishra *et al.,2006*).In Germany, ciprofloxacin resistance rate reached 11.2% in 2003,

increased to 16.6% in 2004, and further increased to 22.1% in 2005 (Glocker,2007). In South Korea, a high resistance rate of 33.8% was reported in strains isolated from adult patients (Kim, 2006). The good susceptibility of *H. pylori* to ciprofloxacin suggests that this antibiotic might be an alternative drug in the regimen treatment for eradication of *H.pylori* in gastro-duodenal ulcerous disease in Anambra state

CONCLUSION

Conclusively, Vegetables cultivated and sold in Odekpe town 6(9.4) could be said to be more contaminated with *H.pylori* than Salad materials 3(3.3) sold in Nnewi town. Therefore, public enlightment programs should be created on the possible risk factors (manure, water sources and animals) of contamination of these products with the organism to reduce microbial load. It is also noteworthy to suggest that while isolating this bacteria from the environment or food sources, several medi, supplemented with a variety of amino-acids or enhancers should be used, including molecular methods, to enhance its culturability. Awareness should be made on the chances of spread of resistant genes of *H.pylori* to other pathogens in feaces and soil and humans can be exposed to these pathogens which have acquired the resistant genes. A form of natural manure process should be bioremediated or treated to avoid the possible spread of these organisms to human.

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