

# International Journal of Advanced Research in Biological Sciences

ISSN: 2348-8069

www.ijarbs.com

DOI: 10.22192/ijarbs

Coden: IJARQG(USA)

Volume 6, Issue 9 -2019

## Research Article



DOI: <http://dx.doi.org/10.22192/ijarbs.2019.06.09.003>

## Antibiotics profile of *Campylobacter jejuni* in surface water in Owerri metropolis.

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### Abstract

Safe water is very important for human health. Contamination of water by microorganisms can be traced to the presence of untreated sewage entering the distribution system. *Campylobacter jejuni* is among the leading causes of gastroenteritis in humans worldwide. The present study was carried out to determine antibiotics profile of *Campylobacter jejuni* in surface in Owerri Metropolis, which include Owerri Municipal, Owerri North and Owerri West. Two hundred and ten (210) samples were collected and analysed using standard methods. The isolates were characterized and the data obtained analyzed using the international web based software (ABIS Online) built with standard Bergey's manual identification procedures. Antimicrobial activity against the isolates was carried by the standard disk diffusion technique based on the recommendation of Clinical Laboratory Standards Institute. The physicochemical properties of the water samples were also analysed. For Heterotrophic bacteria count, result shows that Owerri Municipal had the highest  $(4.8 (10^7) \pm 1.04 \text{ CFU/ml})$ , while the lowest was recorded in Owerri West L.G.A with value of  $5.8(10^5) \pm 0.94 \text{ CFU/ml}$ . *Campylobacter* count, results shows that Owerri Municipal had a significant ( $p < 0.05$ ) higher growth of  $2.8 (10^3) \pm 0.94 \text{ CFU/ml}$ . The antibiotic susceptibility pattern of *Campylobacter jejuni* indicated varied degree of susceptibility to common antibiotics administered. Gentamycin (93.8%), ciprofloxacin (87.5%) and levofloxacin (87.5%) were the most effective drugs for the management of *Campylobacter jejuni* while the bacteria had poor susceptibility to zinicicef. Physicochemical parameters such as turbidity, TDS, phosphate, nitrate, TSS, calcium, COD, BOD, temperature, conductivity, pH, PAH, sulphate, sodium and alkalinity of the samples were analysed. The pH value ranges from 7.13 to 7.73 for the test samples. The pH values of all the samples fall within the WHO permissible limit of 6.5-8.0. Turbidity value of the various water bodies ranged from  $8.11 \pm 0.97$  to  $26.16 \pm 0.34$ . NTU for surface water collected from Owerri Municipal has the highest value of  $26.16 \pm 0.34$  which is above the WHO standard of  $< 25 \text{ NTU}$ . It is important to continue monitoring the water quality of the surface water from the study area as to assess trends in pollution using trace elements, microbiological, physical and chemical as indicators of the behaviours.

**Keywords:** Safe water, *Campylobacter jejuni*, physicochemical properties, antibiotic susceptibility pattern.

### Introduction

Safe drinking water is essential for human health. Contaminated drinking water has the potentials to exert serious health concerns (WHO, 2004). The association between water quality and disease has been recognized for more than a hundred years but till

today the transmission of waterborne diseases is a major public health concern (Hrudey and Hrudey, 2007; NRC, 2004; Theron and Cloete, 2002). The populations of the industrialized nations take the availability of clean water for granted but the need for safeguarding the wholesome good quality of potable water requires continuous vigilance from water supply

companies and this is regulated by public health officers (Percival *et al.*, 2000). Contamination of water can be traced primarily to the presence of animal or human faeces that may originate from untreated sewage entering the distribution system, from animal waste being carried by rain runoff or by melting snow, or from failure or breakdown in the water treatment process (Percival *et al.*, 2000). In many developing countries, faecal contamination of drinking water is a reality. (WHO, 2004) but in industrialized countries, there are also incidence of faecal contamination (Neumann *et al.*, 2005). In fact, in developed countries, many outbreaks of waterborne gastrointestinal illnesses have been traced to water supplies where the drinking water is usually assumed to be of good quality (Hrudey and Hrudey, 2007). The contamination may take place at the water source (reservoir), water treatment plant or within the distribution system. Pathogenic microbes (bacteria, viruses, protozoans, and helminthes) may be present in human or animal faeces and waterborne infections are highly probable in cases when there are failures to prevent faecal contamination of drinking water. Faecal contamination of water may lead to a serious outbreak of diseases since the drinking water distribution networks usually serve a large number of individuals. *Campylobacter* is the most common cause of bacterial gastroenteritis in most parts of the world and *Campylobacter jejuni* is the predominant pathogen (Curtis *et al.*, 2014). Warm-blooded animals, such as poultry, pigs and ruminants are major reservoirs for *Campylobacter* and the bacteria are thought to be mainly transmitted through handling and eating raw or undercooked meat (Thomas, *et al.*, 1998; Skirrow, 1991; Braideet *al.*, 2017; Adeleyeet *al.*, 2018). *Campylobacter* can also be transmitted through environmental pathways, such as water, and waterborne outbreaks of *Campylobacter* are common (Schoenberg-Norio, 2004). Groundwater for drinking is usually not treated in the Nordic countries, and reports from Finland describe *Campylobacter* outbreaks where there is heavy rain which has led to contamination of groundwater wells (Guzman-Herradoret *al.*, 2015). Studies have shown that cattle drinking untreated water from lakes or private water supplies are more likely to test positive for *Campylobacter* (Ellis-Iversen *et al.*, 2009). *Campylobacter jejuni* water survival time at a low temperature varies between two weeks and four months (Chan *et al.*, 2001; Triguier *et al.*, 2015) and strains of *Campylobacter* isolated from different sources have shown different survival potentials (Triguier *et al.*, 2015). These inter-strain differences have

been suggested to be caused by variations in genetic content (Chan *et al.*, 2001).

This study evaluates the antibiotics profile of *Campylobacter jejuni* in surface water in Owerri metropolis.

## Materials and Methods

### Study Area

Owerri is the capital of Imo State in Nigeria and is situated in the heart of Igbo land. It is the State's largest city, followed by Orlu and Okigwe as second and third respectively. Owerri consists of three Local Government Areas including Owerri Municipal, Owerri North and Owerri West; it has an estimated population of about 401,873 as of 2006 census, (Alex, 2008) and is approximately 100 square kilometres (40 sq mt) in area. Owerri is bordered by the Otamiri River to the east and the Nworie River to the south.

#### Sampling points

Two (2) sampling points considered for the study includes; underground and surface water sample. Surface water samples were collected from Nworie, Okatankwo and Otamiri rivers across the three Local Government Area. Surface water samples were collected 200 meters upstream.

### Sample collection

Samples were collected by the method described by Taiwoet *al.* (2014) and Hassan *et al.* (2014). Samples were collected into pre-sterilized screw cap bottles and labelled appropriately. A total of two hundred and ten (210) samples were collected randomly from different points across the different locations. Seventy (70) samples were collected from each Local Government Area.

### Sample preparation and inoculation

A ten-fold serial dilution was adopted as described by Cheesbrough (2009). An aliquot (0.1 ml) of dilutions  $10^{-2}$  samples were plated out in triplicates on a surface dried Nutrient agar, MacConkey Agar, Eosin Methylene Blue (EMB) (Titan Biotech, India) agar and *Campylobacter* Blood Free Agar base (Oxoid, England). Nutrient agar, Eosin Methylene Blue (EMB) and MacConkey agar was incubated aerobically at 37°C for 24 hours while *Campylobacter* agar was incubated in microaerophilic condition for 48 hours.

The colonies formed on each plate were counted and the average counts were recorded as colony forming units per ml (CFU/ml) of the water sample.

### Pure culture technique

Discrete colonies formed after incubation was sub-cultured onto the surface of a dried nutrient agar. Pure cultures obtained was inoculated into 10 ml of Tryptic Soy Broth and stored in the refrigerator for further use.

### Bacterial identification

The identification of bacterial isolates was based on colonial and selected biochemical tests and data obtained are analyzed using the international web based software (ABIS Online) built with standard Bergey's Manual identification procedures. The software is available at [http://www.tgw1916.net/bacteria\\_logare\\_desktop.htm](http://www.tgw1916.net/bacteria_logare_desktop.htm). To identify the organisms, selected biochemical tests were conducted and the result was fed into the software.

### Selected target: *Campylobacter* sp

Recommended tests (best for identification) for *Campylobacter* sp include; urease, indoxyl hydrolysis, hippurate hydrolysis, nitrates, selenite, H<sub>2</sub>S, growth at 25°C and 42°C, oxidase, 2% NaCl, nalidixic acid, Sodium Fluoride (NaF), safranin, alkaline phosphatase activity (pal), cefoperazone, cephalotin, and MacConkey.

### Preparation of 0.5 McFarland solutions

This was prepared according Cheesbrough (2004). One gram (1 g) of Barium chloride, was weighed out and dissolved in 100 ml of water. One milliliter (1 ml) of concentrated sulphuric acid (H<sub>2</sub>SO<sub>4</sub>) was added to 99 ml of water. 0.05 ml of 1% BaCl<sub>2</sub> was mixed with 99.5 ml of 1% H<sub>2</sub>SO<sub>4</sub>. The prepared solution was mixed to form a turbid suspension. The broth cultures were standardized by diluting the broth with sterile water until turbidity equivalent 0.5 McFarland solutions was obtain.

### Antibiotics susceptibility test of the isolates

The antibiotics susceptibility test for the isolates was done on Mueller-Hinton agar using the standard disk diffusion technique based on the recommendation of Clinical Laboratory Standards Institute (CLSI, 2014).

Colonies of 18–24 h old culture was picked and suspended in a tube containing sterile normal saline (0.85% NaCl) and the turbidity adjusted to 0.5 (McFarland standards). With the aid of a sterile swab stick, the suspension was uniformly spread over already prepared Mueller Hinton agar plates and the antibiotics were placed carefully on the plates with the aid of sterile forceps. The plates were inverted and incubated at 37°C for 18–24 h. Zones of inhibition were measured, recorded and compared/interpreted to the CLSI standards (CLSI, 2014). The results were categorized as: R (resistant) and S (sensitive).

### Physiochemical parameter analysis

Under listed parameters were determined using standard methods according to APHA, (2017). The physicochemical properties determined include Temperature, pH, Turbidity, Dissolved Oxygen (DO), Total alkalinity, Acidity, Conductivity, Total hardness, Total Dissolved Solids (TDS), Total Suspended Solids (TSS), Total Solids (TS), Chemical Oxygen Demand (COD), Biochemical Oxygen Demand (BOD), Salinity, Nitrate, and PO<sub>4</sub><sup>3-</sup>. Presence of minerals (such as magnesium and calcium) was determined by methods EDTA Titration method.

## Results

Microbial enumeration of the surface water samples. Table 1 shows the enumeration and distribution of microorganisms from surface water sampled in the three Local Government Areas of Owerri. For Heterotrophic bacteria count, result shows that Owerri Municipal had the highest ( $4.8 \times 10^7 \pm 1.04$  CFU/ml). Analysis of variance shows that there was significant difference ( $p < 0.05$ ) among the Local Governments Areas examined for heterotrophic bacterial count in surface water samples. For total coliform count, ANOVA shows that surface water collected from Owerri Municipal had a significant ( $p < 0.05$ ) higher coliform count of  $7.1 \times 10^4$  CFU/ml as against Owerri North ( $8.0 (10^2) \pm 0.87$  CFU/ml) and Owerri West ( $7.0 \times 10^4 \pm 0.86$  CFU/ml), respectively. For total *Campylobacter* count, results shows that Owerri Municipal had a significant ( $p < 0.05$ ) higher growth of  $2.8 \times 10^3 \pm 0.94$  CFU/ml.

**Table 1: Microbial Load of Underground Water and Surface Water samples**

Parentthesis	Owerri Municipal n=210	Owerri North n=210	Owerri West n=210
	Surface Water n=70	Surface Water n=70	Surface Water n=70
	Mean $\pm$ SEM CFU/ ml	Mean $\pm$ SEM CFU/ ml	Mean $\pm$ SEM CFU/ ml
THBC	4.8(10 <sup>7</sup> ) $\pm$ 1.04	1.4(10 <sup>7</sup> ) $\pm$ 1.04	5.8(10 <sup>5</sup> ) $\pm$ 0.94
TCC	7.1(10 <sup>4</sup> ) $\pm$ 0.94	8.0 (10 <sup>2</sup> ) $\pm$ 0.87	7.0 (10 <sup>4</sup> ) $\pm$ 0.87
TC <sub>b</sub> C	2.8(10 <sup>3</sup> ) $\pm$ 0.94	7.5(10 <sup>1</sup> ) $\pm$ 0.33	1.5(10 <sup>2</sup> ) $\pm$ 0.13

KEY: THBC = Total Heterotrophic Bacterial count; TCC = Total Coliform count; TC<sub>b</sub>C = Total *Campylobacter* count; CFU = Colony Forming unit

### Identification of bacterial isolates

The isolates were identified based on morphological, microscopic and biochemical characterization. The data obtained was analyzed using the international web based software (ABIS Online) built with standard Bergey's Manual identification procedures. The biochemical tests result is shown in Table 2. Data obtained was fed into the ABIS online software and results obtained were displayed in Figure 1. The isolate identified was *Campylobacter jejuni*. Eleven (11) other bacteria isolated from the surface water

includes; *Escherichia coli* and *Micrococcus*, *Bacillus*, *Klebsiella*, *Citrobacter*, *Pseudomonas*, *Staphylococcus*, *Salmonella*, *Vibrio*, *Proteus*, and *Enterobacter* species. The morphology and biochemical characterization of these isolates is shown in Table 3. Figure 1 showed a degree of relatedness of the isolates identified by the software to have similar biochemical tests. Results indicated that the isolate (F) was closely related to *Campylobacter jejuni* subsp. *doylei* by 99 % compared to *Helicobacterfennelliae*(92%), *Helicobacter canis*(81%) and *Campylobacter helveticus*(76%).

**Table 2: Biochemical characterization of the isolates.**

<i>Campylobacter</i> sp	
Test	Result
Indoxyl hydrolysis	-
Nalidixic acid	-
Hippurate hydrolysis	+
NaF	-
Nitrates	-
Safranin	-
Selenite	-
PAL	+
H <sub>2</sub> S	-
Cefoperazone	-
Oxidase	+
2% NaCl	-
Cephalotin	-

Key: NaF= Sodium Fluoride, PAL= Alkaline phosphatase activity, H<sub>2</sub>S= Hydrogen sulphide, +=Positive, - = Negative.

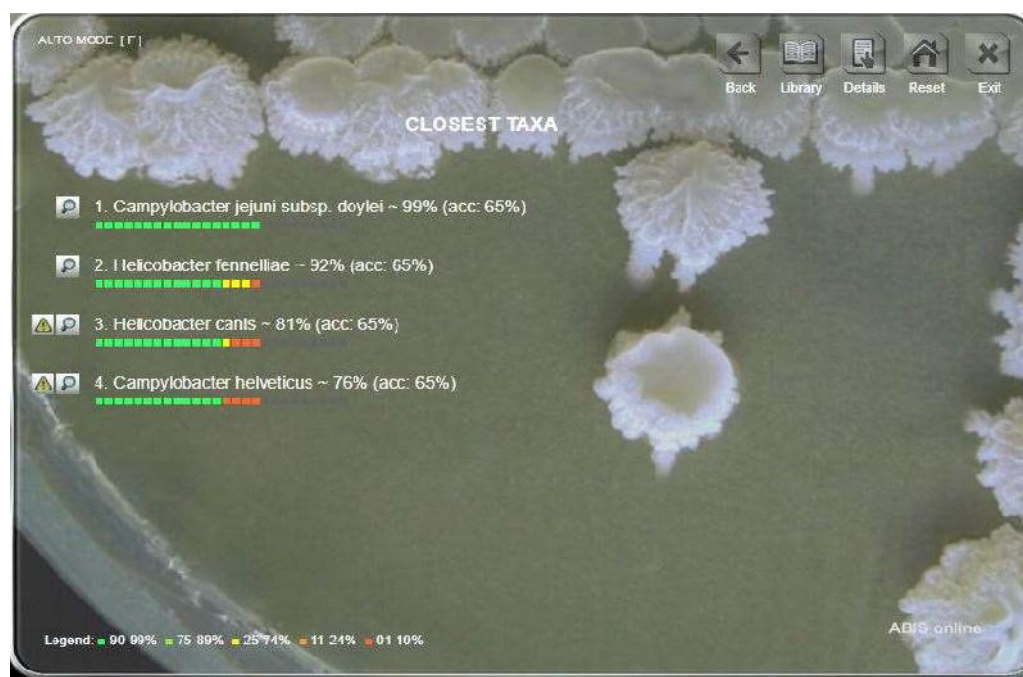
**Table 3: Identification of Bacterial Isolates from Surface Water.**

Bacterial isolates	Gram reaction	Cellular morphology	Motility	Catalase	Oxidase	Indole	Nitrate reduction	Methyl red	Voges proskauer	Oxidation fermentation	Ornithine decarboxylase	PAL	H <sub>2</sub> S	Cefoperazone	Cephalotin	2% NaCl	NaF	Nitrates	Safranin	Selenite	Hippurate hydrolysis	Indoxyl hydrolysis	Urease	Spore forming	Oxygen utilization	Probable organism
B1	-	R	+	+	-	-	+	-	+	F	+	-	-	-	-	-	-	-	-	-	-	-	-	-	A	<i>Enterobactersp.</i>
B2	+	R	-	+	-	-	-	-	+	O	+	-	-	-	-	-	-	-	-	-	-	-	-	+	A	<i>Bacillus sp.</i>
B3		R	+	+	-	-	+	+	-		-	-	-	-	-	-	-	-	-	-	-	-	-	-	A	<i>Salmonella sp.</i>
B4		R	+	-	+	-	+	-	-	F	-	-	-	-	-	-	-	-	-	-	-	-	-	-	Fa	<i>Pseudomonas sp.</i>
B5		R	+	+	-	+	+	+	-	F	+	-	-	-	-	-	-	-	-	-	-	-	-	+	A	<i>Escherichia sp.</i>
B6		R	+		+	+	+	-			-	-	-	-	-	-	-	-	-	-	-	-	-	-	A	<i>Vibrio sp.</i>
B7	+	C	+	+	-	+	-	-	-	O	-	-	-	-	-	-	-	-	-	-	-	-	-	-	A	<i>Micrococcus sp.</i>
B8	-	R	-	+	-	-	+	-	+	F	-	-	-	-	-	-	-	-	-	-	-	-	+	-	A	<i>Klebsiellasp.</i>
B9	-	R	+	+	-	-	+	+	-	F	-	-	-	-	-	-	-	-	-	-	-	-	-	-	Fa	<i>Citrobactersp.</i>
B10	-	R	+	+	-	+	+	+	-	F	+	-	-	-	-	-	-	-	-	-	-	-	-	+	A	<i>Escherichia sp.</i>
B11	-	R	-	-	+	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	A	<i>Campylobacter sp.</i>
B12	-	R	+	+	-	-	+	+	-	O	+	-	-	-	-	-	-	-	-	-	-	-	+	-	A	<i>Proteus sp.</i>

Key:

**-ve: Negative,****Fa: facultative anaerobic****A: Aerobic****+ve: Positive,****O: oxidative****C: Coccus****F: fermentative****R: Rod****NaF: Sodium Fluoride****PAL: Alkaline phosphatase****H<sub>2</sub>S: Hydrogen sulphide****+: Positive****- : Negative.****B1-D12: Bacterial Isolates from underground water**



Figure 1: ABIS Online results for *Campylobacter jejuni*.

#### Percentage occurrence of bacterial isolates from surface water in the area studied

Table 4 shows the percentage occurrence of bacterial isolated from surface water samples from three Local Government Areas studied. Out of the 207 different bacterial isolates, 14(6.76%) *Micrococcus* sp., 13(6.28%) *Bacillus* sp., 13(6.28%) *Citrobactersp.*, 12(5.80%) *Pseudomonas* sp., 40(19.32%) *Escherichiacoli*, 15(7.25%) *Staphylococcus* sp., 16(7.73%) *Campylobacter* sp., 33(15.94%)

*Klebsiellasp.*, 12(5.80%) *Salmonella* sp., 14(6.76%) *Proteus* sp., 13(6.28%) *Enterobactersp* and *Vibrio* sp. 12(5.80%). Analysis of variance shows that *Escherichia coli* were the most statistically ( $p < 0.05$ ) predominant isolate isolated from the surface water from the three local government areas. *Pseudomonas*, *Salmonella* and *Vibrio* species were the least isolated bacterial in the study area. Analysis shows that Owerri Municipal had a significant higher number of isolates as against Owerri North and Owerri West, respectively ( $F=3.025$   $r^2=0.01939$ ,  $P$  value=0.0068).

Table 4: Percentage occurrence of bacterial isolates from surface water in the area studied

Isolates	Owerri municipal (%)	Owerri North (%)	Owerri West (%)	Total (%)
<i>Micrococcus</i> sp.	5(2.42)	6(2.90)	3(1.45)	14(6.76)
<i>Bacillus</i> sp.	6(2.90)	3(1.45)	4(1.93)	13(6.28)
<i>Citrobactersp.</i>	4(1.93)	5(2.42)	4(1.93)	13(6.28)
<i>Pseudomonas</i> sp.	6(2.90)	2(0.97)	4(1.93)	12(5.80)
<i>Escherichia</i> sp.	17(8.21)	10(4.83)	13(6.28)	40(19.32)
<i>Staphylococcus</i> sp.	5(2.42)	4(1.93)	6(2.90)	15(7.25)
<i>Campylobacter</i> sp.	7(3.38)	5(2.42)	4(1.93)	16(7.73)
<i>Klebsiellasp.</i> ,	14(6.76)	11(5.31)	8(3.86)	33(15.94)
<i>Salmonella</i> sp.	4(1.93)	3(1.45)	5(2.42)	12(5.80)
<i>Proteus</i> sp.,	5(2.42)	4(1.93)	5(2.42)	14(6.76)
<i>Enterobactersp.</i>	4(1.93)	5(2.42)	4(1.93)	13(6.28)
<i>Vibrio</i> sp.	4(1.93)	3(1.45)	5(2.42)	12(5.80)
TOTAL	81(39.13)	61(29.47)	65(21.17)	207(100)

### Physicochemical Properties of Surface Water collected from three Local Government Area in Owerri, Imo State

Table 5 shows the result of physicochemical parameters such as turbidity, TDS, phosphate, nitrate, TSS, calcium, COD, BOD, temperature, conductivity, pH, PAH, sulphate, sodium and alkalinity of the samples. The pH value (hydrogen ion concentration) ranges from 7.13 to 7.73 for the test samples. The pH values of all the samples fall within the WHO permissible limit of 6.5-8.0. Data shows that TSS and TDS of water bodies sampled were all within the maximum permissible limit as per the WHO standards (<500mg/L and >50 mg/L), respectively for portable water. Tukey's post *hoc* test for multiple comparisons showed that TSS and TDS values from Owerri North were significant higher ( $p < 0.05$ ) than that of Owerri West and Owerri Municipal.

Water temperature was not within the limit ( $25^{\circ}\text{C}$ ) among the surface water collected from the three Local Government Areas. The values were between  $25.42 \pm 0.98$  and  $33.00 \pm 1.03$ . Analysis of variance indicates that there was no significant difference ( $p < 0.05$ ) in the water temperature in the different local government area, although surface water samples collected from Owerri West had a significant ( $p < 0.05$ ) higher temperature as against Owerri North and Owerri Municipal.

Turbidity value of the various water bodies ranged from  $8.11 \pm 0.97$  to  $26.16 \pm 0.34$  NTU for surface water collected from Owerri Municipal has the highest value of  $26.16 \pm 0.34$  which is above the WHO standard of <25NTU.

The alkalinity values of the surface water examined in the three local government areas ranges from  $25.14 \pm 1.11$  to  $61.00 \pm 1.23$  mg/L. The alkalinity values of the various water bodies fall within the WHO standard (<500mg/L) in all the samples examined. Generally, surface water collected from Owerri North showed higher value ( $61.00 \pm 1.23$ mg/L) but was not significant ( $p < 0.05$ ) when compared with the water samples in other Local Government Areas. Chemical oxygen demand (COD) of the samples from the three Local Government Areas ( $109.20 \pm 2.65$  to  $186.20 \pm 1.56$ ) falls within the WHO standard of <100 to 300 mg/L.

The values of conductivity from the surface water samples collected from the three LGAs vary in the different samples and fall within the criteria (<1000 $\mu\text{S}/\text{cm}$ ) set by the World Health Organization for portable water but below the limit set by FAO for irrigation, indicating a high amount of dissolved inorganic substances in their ionizing form.

Table 5: Physicochemical properties of the water samples

Parameters	Surface water			WHO Standard
	Owerri Municipal MEAN $\pm$ SEM n=70	Owerri North MEAN $\pm$ SEM n=70	Owerri West MEAN $\pm$ SEM n=70	
Turbidity (NTU)	$26.16 \pm 0.34$	$18.05 \pm 0.34$	$14.56 \pm 0.67$	<25
Total Dissolved Solid (mg/L)	$505.1 \pm 1.24$	$523.1 \pm 1.32$	$521.1 \pm 2.34$	>5.0
Phosphate (mg/L/mg/kg)	$1.62 \pm 0.45$	$3.62 \pm 0.53$	$2.62 \pm 0.71$	5.0
Nitrate (mg/L/ mg/ kg)	$21.54 \pm 1.12$	$27.41 \pm 1.04$	$29.14 \pm 1.32$	45
Alkalinity (mg/L)	$51.10 \pm 2.31$	$61.00 \pm 1.23$	$43.00 \pm 2.12$	30-500
Total Suspended Solid (mg/L)	$214.00 \pm 1.28$	$256.00 \pm 2.34$	$261.10 \pm 1.81$	<500
COD (mg/L/mg/kg)	$109.20 \pm 2.65$	$114 \pm 2.11$	$142.60 \pm 1.65$	<100 to 300
BOD (mg/L/mg/kg)	$981.50 \pm 2.54$	$972.40 \pm 1.89$	$962.42 \pm 2.01$	28-30
Temperature ( $^{\circ}\text{C}$ )	$29.00 \pm 1.27$	$31.00 \pm 0.86$	$33.00 \pm 1.03$	< $25^{\circ}\text{C}$
Conductivity ( $\mu\text{S}/\text{cm}$ )	$862.40 \pm 2.41$	$816.21 \pm 1.85$	$872.73 \pm 2.87$	<1000
pH	$7.42 \pm 0.12$	$7.17 \pm 0.32$	$7.65 \pm 0.45$	6.5-8.0
Sulphate (mg/L/mg/kg)	$299.04 \pm 1.23$	$298.56 \pm 0.43$	$309.12 \pm 2.67$	500
Sodium (mg/L/mg/kg)	$7.48 \pm 0.75$	$7.68 \pm 0.13$	$7.73 \pm 0.45$	<50
Calcium (mg/L/mg/kg)	$0.824 \pm 0.01$	$0.914 \pm 0.00$	$0.784 \pm 0.00$	> 5 (2.5)

KEY: T= Test sample; C= Control sample; COD= Chemical Oxygen Demand; BOD= Biochemical Oxygen Demand

**Table 6:Antibiogram of Bacterial Isolates fromSurface Water samples collected from the Sample Area**

<b>Organisms</b>	<b>No. (%) of Isolates</b>	<b>PERCENTAGE OF BACTERIA SUSCEPTIBLE TO:</b>												
		<b>AML</b>	<b>RD</b>	<b>CH</b>	<b>AU</b>	<b>CEP</b>	<b>SXT</b>	<b>CPX</b>	<b>CN</b>	<b>S</b>	<b>LEV</b>	<b>TET</b>	<b>NAL</b>	<b>Z</b>
		<b>30µg</b>	<b>30µg</b>	<b>30µg</b>	<b>25µg</b>	<b>30µg</b>	<b>30µg</b>	<b>30µg</b>	<b>10µg</b>	<b>30µg</b>	<b>30µg</b>	<b>25µg</b>	<b>30µg</b>	<b>25µg</b>
<i>Micrococcus</i> sp.	21(8.7)	19(90.5)	8(38.1)	9(42.9)	20(95.2)	10(47.6)	10(47.6)	18(85.7)	10(47.6)	8(38.1)	14(66.7)	8(38.1)	10(47.6)	12(57.1)
<i>Bacillus</i> sp.	18(7.4)	9(50.0)	12(66.7)	6(33.3)	15(83.3)	15(83.3)	12(66.7)	15(83.3)	9(50.0)	6(33.3)	12(66.7)	9(50.0)	9(50.0)	12(66.7)
<i>Citrobactersp.</i>	17(7.0)	12(70.6)	12(70.6)	12(70.6)	12(70.6)	12(70.6)	8(47.1)	16(94.1)	12(70.6)	8(47.1)	12(70.6)	12(70.6)	8(47.1)	16(94.1)
<i>Pseudomonas</i> sp.	14(5.8)	6(42.9)	9(64.3)	6(42.9)	12(85.7)	12(85.7)	8(57.1)	12(85.7)	9(64.3)	8(57.1)	12(85.7)	0(0.0)	6(42.9)	12(85.7)
<i>Escherichia</i> sp.	48(19.8)	30(62.5)	20(41.7)	30(62.5)	41(85.4)	30(62.5)	30(62.5)	30(62.5)	32(66.7)	31(64.6)	41(85.4)	30(62.5)	22(45.8)	33(68.8)
<i>Staphylococcus</i> sp.	19(7.85)	14(73.7)	14(73.7)	14(73.7)	16(84.2)	14(73.7)	13(68.4)	15(78.9)	14(73.7)	15(78.9)	15(78.9)	15(78.9)	14(73.7)	16(84.2)
<i>Campylobacter</i> sp.	21(8.7)	13(61.9)	12(57.1)	12(57.1)	13(61.9)	13(61.9)	12(57.1)	19(90.5)	18(85.7)	12(57.1)	18(85.7)	12(57.1)	11(52.4)	12(57.1)
<i>Klebsiellasp.,</i>	33(13.6)	24(72.7)	24(72.7)	23(69.7)	24(72.7)	25(75.8)	24(72.7)	24(72.7)	23(69.7)	28(84.8)	15(45.5)	19(57.6)	12(36.4)	14(42.4)
<i>Salmonella</i> sp.	12(5.0)	6(50.0)	7(58.3)	4(33.3)	6(50)	6(50)	7(58.3)	6(50.0)	8(66.7)	4(33.3)	8(66.7)	4(33.3)	2(16.7)	10(83.33 )
<i>Proteus</i> sp.,	14(5.8)	6(42.9)	3(21.4)	6(42.9)	9(64.3)	9(64.3)	9(64.3)	6(42.9)	6(42.9)	6(42.9)	9(64.3)	6(42.9)	6(42.9)	6(42.9)
<i>Enterobactersp.</i>	13(5.4)	6(46.2)	6(46.2)	9(69.2)	12(92.3)	9(69.2)	9(69.2)	9(69.2)	3(23.1)	6(46.2)	9(69.2)	6(46.2)	9(69.2)	6(46.2)
<i>Vibrio</i> sp.	12(5.0)	6(50.0)	4(33.3)	5(41.7)	5(41.7)	4(33.3)	6(50.0)	6(50.0)	6(50.0)	6(50.0)	6(50.0)	0(0.0)	4(33.3)	6(50.0)
Total	242(100)	151(62.4)	131(54.1 )	136(56.2 )	185(76.4 )	159(65.7 )	148(61.2 )	176(72.7 )	150(62.0 )	138(57.0 )	171(70.7 )	121(50.0 )	113(46.7 )	155(64.1 )

**AML= Amoxil, RD=Rifampicin, CH=Chloramphenicol, AU=Augmentin, CEP=Ceporex, SXT=Seprine, CPX=Ciproflox, CN=Gentamycin, S=Streptomycin, LEV=Levofloxacin, TET=Tetracycline, NAL=Nalidixic Acid, Z=Zincef.**



### Antibiogram of Bacterial Isolates from Surface Water

Results of susceptibility testing on isolates from the surface water bodies collected from the three LGAs in Owerri are summarized in Table 6. The antibiogram of bacterial isolates indicated varied degree of susceptibility to common antibiotics administered in the study. Augmentin (76.4%), ciprofloxacin (72.7%), levofloxacin (70.7%) and ceporex (65.7%) were the most effective drugs for the management of bacterial isolated from the water bodies sampled. None of the isolates had 100% susceptibility to the bacterial isolates. While all the bacteria had poor susceptibility to nalidixic acid (46.7%), Tetracycline (50.0%), Rifampicin (54.1%) and Streptomycin (57.0%).

### Antibiotic sensitivity pattern of *Campylobacter jejuni* Isolated from Surface water from the Study Area

The antibiotic susceptibility pattern of *Campylobacter jejuni* isolated from the surface water in the three LGAs examined in Owerri indicated varied degree of susceptibility to common antibiotics administered in the study; gentamycin (93.8%), ciprofloxacin (87.5%) and levofloxacin (87.5%) were the most effective drugs for the management of *Campylobacter jejuni* while the bacteria had poor susceptibility from the surface water bodies collected from the three LGAs in Owerri studied (Fig 2).

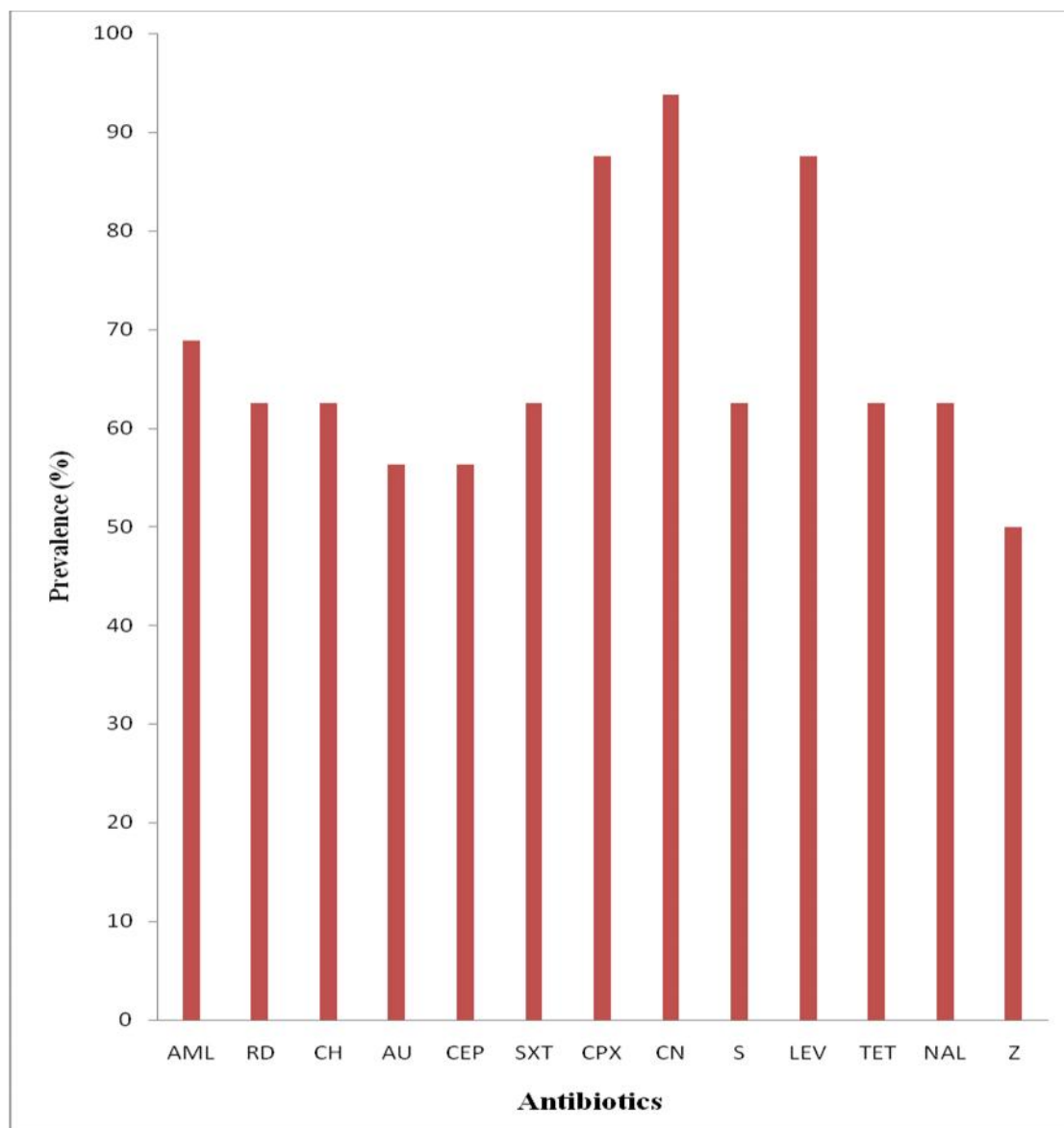


Figure 2: Antibiotic sensitivity pattern of *Campylobacter jejuni* isolated from Surface water from the study area

## Discussion

The quality of water is determined by its physical, chemical and biological properties which are influenced by a host of natural and human factors. The natural factors are geological, hydrological and climatological while human factors include pollution activities such as discharge of domestic, anthropogenic, industrial, urban and other waste waters and the disposal of chemicals into drainage basin (Bartam and Balance, 1996). The result has shown the presence of pathogenic and indicator microorganisms. Result shows the presence of seven (7) microorganisms. They include; *Micrococcus* sp. 7 (20.00%), *Bacillus* sp. 5 (14.29%), *Citrobacter* sp. 4 (11.43%), *Pseudomonas* sp. 2 (5.71%), *Escherichia* sp. 8 (22.86%), *Staphylococcus* sp. 4 (11.43%), and *Campylobacter* sp. 5 (14.29%) (Table 3).

Result of the enumeration and distribution of microorganisms from the surface water sampled in the three LGAs of Owerri shows that heterotrophic bacteria count from Owerri Municipal had the highest ( $4.8 (10^7) \pm 1.04$  CFU/ml) in surface water. Analysis of variance shows that there were significant difference ( $p < 0.05$ ) among the LGAs examined for heterotrophic bacterial count. For total coliform count, ANOVA shows that the samples collected from Owerri Municipal had a significant ( $p < 0.05$ ) higher coliform count of  $7.1 \times 10^4$  CFU/ml as against Owerri North ( $8.0 (10^2) \pm 0.87$  CFU/ml) and Owerri West ( $7.0 (10^4) \pm 0.86$  CFU/ml), respectively. For total *Campylobacter* count, results shows that Owerri Municipal had a significant ( $p < 0.05$ ) higher growth of  $2.8 (10^3) \pm 0.94$  CFU/ml.

Water pollution is responsible for the transmission of infectious diseases such as cholera, diarrhoea and typhoid (Nassinyama *et al.*, 2000). For a water to be portable, WHO recommends not more than 10 MPN/100 ml of total coliforms and none for fecal coliforms (WHO, 2006). All the water bodies recorded high number of coliforms which were significantly high in Owerri Municipal. The high level of microbial loads recorded throughout the water bodies is mostly attributed to organic deposits predominantly from human and animal sewerage as well as high suspended solid matter (Madema *et al.*, 2003). The high population and human activities around Owerri Municipal is also an attribute for the high microbial load recorded in Owerri Municipal. Washing of clothes, cars, recreational and anthropogenic activities are other potential sources of faecal contamination.

These results agree with previous studies that showed that waste water from abattoir has great burden of pathogens associated with diarrhoeal infections (Brooks *et al.* 2003; Brooks *et al.* 2006). In a study by Onyuka *et al.* (2011) *S. typhimurium* (49.6%) was the predominant isolate followed by *E. coli* and *V. cholera* with prevalence of 46.6% and 3.8%, respectively. It is therefore concluded that the water samples were contaminated with bacteria at levels that are above the WHO standards for portability.

The antibiotic sensitivity/resistance of bacterial pathogens associated with the water bodies was examined. Some antibiotics were more effective on some organisms than others. Antibiotics also showed varied inhibitory and sensitivity effects on bacterial pathogens isolated from the water bodies. Augmentin (76.4%), ciprofloxacin (72.7%), levofloxacin (70.7%) and cephalexin (65.7%) were the most effective drugs for the management of bacterial isolated from the water bodies sampled. This finding is in agreement with the study by Valdes *et al.* (2005) who reported that ciprofloxacin and augmentin remains the drug of choice for the treatment of infections associated with the bacterial isolated from the present study. Above all, they are broad spectrum antibiotics known to be effective against different type of organisms including Gram positive and Gram negative bacteria. Nalidixic acid (46.7%), Tetracycline (50.0%), Rifampicin (54.1%) and Streptomycin (57.0%) showed low inhibitory effect. This finding is also in agreement with that by Valdes *et al.* (2005). Low inhibitory effect of these antibiotics could be attributed to the differences in the concentrations of antibiotics, source of isolates and drug resistance transfer (Corrigan and Boineau, 2001).

Furthermore, indiscriminate use and abuse of some antibiotics such as septrin, tetracycline and streptomycin could also contribute to the low effect observed. Recent studies demonstrated that domestic vectors play an important role in spreading antibiotic resistant genes among bacteria (Frank *et al.*, 2011).

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#### How to cite this article:

Ogu, N.P., Ettien, C.E and Braide, W. (2019). Antibiotics profile of *Campylobacter jejuni* in surface water in Owerri metropolis. *Int. J. Adv. Res. Biol. Sci.* 6(9): 20-31.

DOI: <http://dx.doi.org/10.22192/ijarbs.2019.06.09.003>