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Evaluation of water quality index and bacteriological qualities of Ossiomo River, Orhionmwon Local Government Area, Edo State, Nigeria

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Abstract
The water quality of Ossiomo river is a great concern due to exposure to industrial contaminants. This study investigates the water quality index (WQI) and bacteriological qualities of Ossiomo river, Orhionmwon Local Government Area, Edo State, Nigeria. Water samples were collected in triplicate from July, 2019 – Dec., 2019, for physiochemical and bacteriological analyses using standard analytical procedures include pour plate and most probable number techniques. Antibiogram test was carried out using Muller-Hinton antibiotic disc diffusion method. Water Quality Index values range from 275.75 – 394.01 mg/l. Total heterotrophic bacterial counts ranged between 2.4±1.1 – 17.8±4.9 ×10³ cfu/ml. Six bacterial isolates were found and identified as Pseudomonas aeruginosa, Escherichia coli, Proteus mirabilis, Shigella sonnei, Salmonella enterica and Klebssiella pnuemoniae. Escherichia coli 19(24) was found to be the most frequent bacterial isolate while Shigella sonnei 11.2(14.2) was least. ciprofloxacin 100%, ceftriaxone 100%, azithromycin 90%, gentamycin 100%, pefloxacin 95%, oflaxacin 75%, cefuroxime 70% and ceftazidine 60% were effective drugs against Enterobacteriaceae infections whereas, augmentin 0% and nitrofurantoin 0% were resistant. There is the need to enforce and implement water quality policy to avoid increase and continuous contamination of Ossiomo River so as to curb bacterial proliferation and water borne diseases within the dependent communities.

Keywords: Antibiogram, Bacteriological, Molecular identification, Ossiomo River, Water Quality Index

Introduction
Human access to clean, safe drinking water is a fundamental human right as declared by the United Nations General Assembly (UN, 2010) and water quality is key to its fulfillment. Portable water is regarded as one of the highest required chemical compounds necessary for life, essential for maintenance of body organs, healthy living as well as survival (Epunde *et al.*, 2017). The earth's surface water is categorized into streams, springs, rivers, ponds, lakes

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as well as reservoirs (Manahan, 2010). Thus, majority of the earth's water sources are derived from precipitation on watershed geographical areas which flows across streams, rivers and occasionally in ponds and lakes hence, the constant changes of moisture content between the earth and atmosphere constitutes the hydrological cycle which primarily form the main source of water for domestic uses, irrigation, production and construction (Khan *et al.,* 2015; Aroor, 2017; Sanjoy *et al.,* 2019). There is a rising global concern about water bodies' degradation as a result of constant exposure to human and atmospheric release of undesirable materials capable of altering the water quality, now considered a vehicle for the spread and dissemination of human associated water borne diseases (Sener *et al.,* 2017; Kumar and Singh, 2018). In addition to anthropogenic activities are natural processes such as channels through which the water flows and interacts with the medium, chemical properties of the river basin and geochemical influences are factors responsible for low water quality, thus presenting it unfit for human consumption, industrial and agricultural uses as well as other vital purposes (Giridharan *et al.,* 2010; Sener *et al.,* 2017). Indiscriminate release of industrial sewage effluents, domestic wastewater, and agricultural runoff are considered as major sources of pollutants of the recipient water body (Barakat *et al.,* 2016).

The increased human population and improved agricultural activities with conscious and unconscious introduction of harmful chemical substances have done serious damages to the quality of most coaster water bodies in the valley as well as Ossiomo river. This study was aimed at evaluating the water quality index and bacteriological quality of Ossiomo River, Orhionmwon Local Government Area, Edo State, Nigeria.

Materials and Methods

Study area

This study was carried out in Ossiomo River (Latitudes $6^0$30'- $6^0$32'0''N; Longitudes $5^0$39'-$5^0$40'30''E), a tributary of Benin River located in Abudu community, Orhionmwon Local Government Area, Edo State, Southern Nigeria. Ossiomo River (Fig 1) stretches over a distance of 250 km within Edo and Delta States, Nigeria.

Sample collection

Water samples were collected in triplicate at monthly interval from July 2019 – Dec. 2019 covering three sampling locations which were station 1 (Upstream), station 2 (Midstream) and station 3 (Downstream). Samples were collected in sterile 1 L screw cap glass bottle and thereafter transported to the laboratory for physicochemical and bacteriological analyses (APHA, 2005).

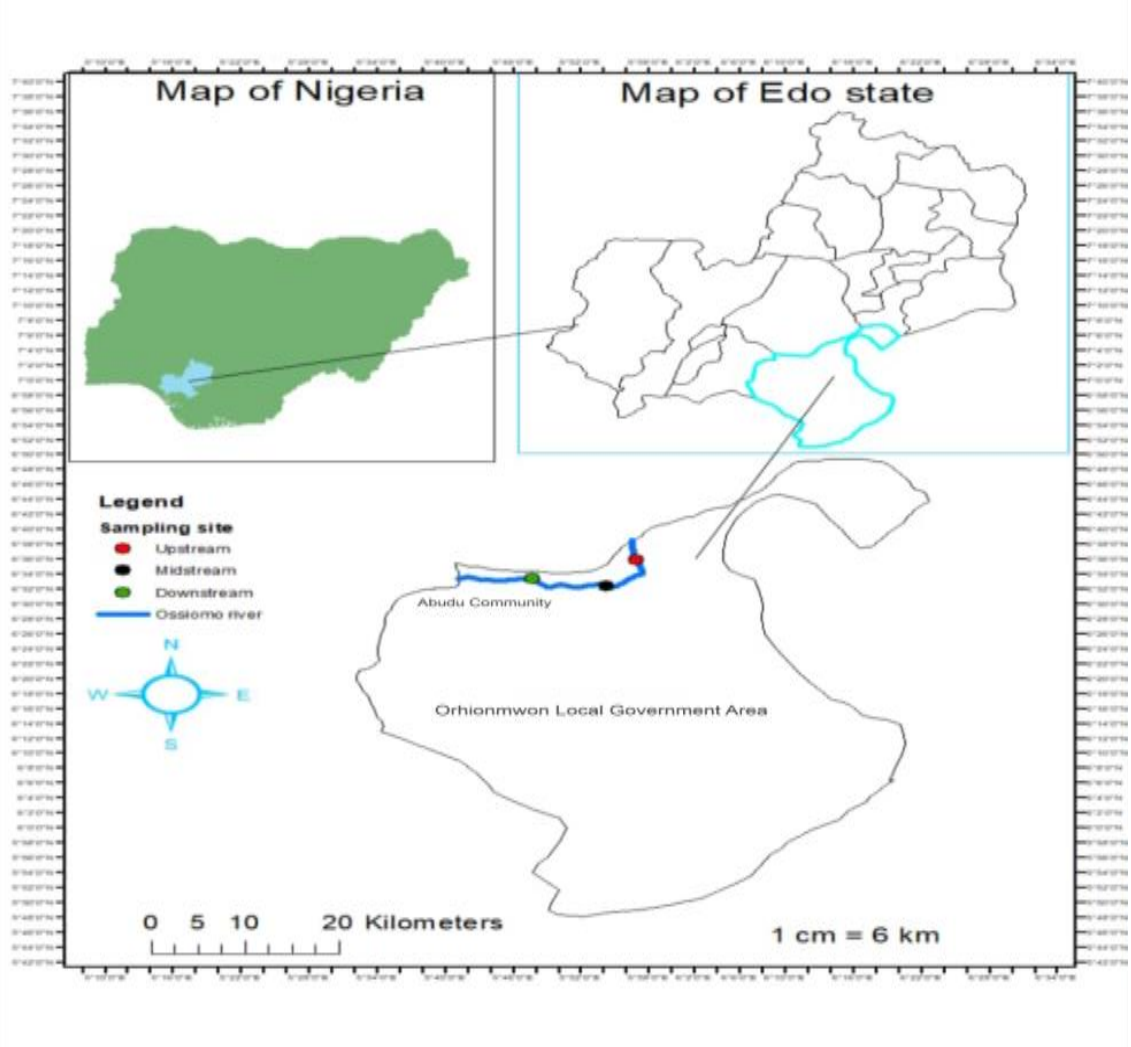

Fig 1: Map of Edo State showing the sampling sites along Ossiomo River stretch

Physicochemical parameters

The water samples were analyzed for selected parameters which included pH, temperature, electrical conductivity, total dissolved solids, Dissolve Oxygen (DO), Biochemical Oxygen Demand (BOD_5), nitrate, sodium, potassium, calcium, magnesium, chloride, phosphorus and sulphate using the standard recommended procedures for water quality monitoring as described by APHA (2005).

Heavy metals

The water samples were analyzed for selected heavy metals such as iron, lead, zinc, cadmium, copper, and chromium using Atomic Absorption Spectrophotometer (900H, Perkin Elmer, Akron, OH, USA) according the methods (APHA, 2005).

Water quality index

The WQI of the sampled river water was assessed using the weighted arithmetic index method as recommended by Bureau of Indian Standards (BIS) (Table 1A and B), Indian Council for Medical Research (ICMR) and World Health Organization (WHO) (ICMR, 1975; WHO, 2011; Bharti and Katyal, 2012). Chemical parameters such as pH, electric conductivity, temperature, total dissolved solids, bicarbonate, sodium, calcium, magnesium, iron and zinc were used to determine the water quality index of Ossiomo River.

Weighted (*wi*) chemical parameters were assigned with respect to their relative importance in quality of drinking water and were computed using the following equations;

$$Wi = \frac{wi}{\sum_{i=1} wi} \quad \text{(i)}$$

Where;

W*i* is the relative weight
wi is the weight of each parameter and
n is the number of parameter.

$$qi = \left(\frac{ci}{si}\right) \times 100 \quad \text{(ii)}$$

Where,

q*i* is the quality rating
C*i* is the concentration of each chemical parameter in each water sample in mg/l and Si is the index drinking water standard for each chemical parameter in mg/l

$$sli = wi \times qi \quad \text{(iii)}$$
$$WQI = sli$$

Where,

Sl*i* is the sub-index of i^{th} parameter
W*i* is the relative weight of i^{th} parameter
q*i* is the rating based on concentration of i^{th} parameter

Source: WHO, 2011; BIS, 2012

Bacteriological analyses of sampled water: Isolation and enumeration of total heterotrophic bacterial isolates were carried out using Nutrient agar, MacConkey agar and Eosin Methylene Blue agar adopting pour plate technique while fecal and total coliforms were determined using Most Probable Number (MPN) according to Cheesebrough (2006). Plates were incubated at 37^0C for 24 hours and discrete colonies were counted and recorded as colony forming unit per milliliter (cfu/ml). Pure cultures were stored in Nutrient agar slant at 4^0C for further characterization and identified using the taxonomic scheme of Bergey's manual of determinative bacteriology (Harley & Prescott, 2002; Cheesebrough, 2006; Sharma, 2009).

Antibiotic susceptibility test of bacterial isolates: The antibiotic susceptibility test for each isolate was performed on freshly prepared, dry surfaced Mueller Hinton agar (Oxoid) using the agar-disk diffusion method according to Clinical Laboratory Standard Institute (CLSI, 2002). A total of eleven (11) tested antibiotics disc (Oxiom) were employed and they are augmentin (30 µg), ceftriaxone (30 µg), nitrofurantoin (300 µg), ofloxacin (5 µg), azithromycin (15 µg), cefuroxime (30 µg), gentamicin (10 µg), ciprofloxacin (5 µg), levofloxacin (5 µg), pefloxacin (5 µg) and ceftazidime (30 µg). The ranges of the diameter were measured in millimeter (mm) of each antibiotic disc which interprets for susceptibility, intermediate or resistance comparing with the performance standards of CLSI (2008).

Results and Discussion

The results (Table 2) obtained from the Water Quality Index for different sampling locations across the periods ranged from 275.75 – 394.01 mg/l (upstream and downstream). Upstream

sample is rated very poor water quality while midstream and downstream samples were rated unsuitable for drinking purposes. This may be due to the influence of anthropogenic activities as well as direct dumping of waste, industrial contaminants and agricultural runoffs into the receiving water body. These findings were in compliance with the study of Singh and Hussian (2016), on assessment of greater Noida Sub-basin, Uttar Pradesh, India, who attributed the presence of toxic chemicals in the water body to human and industrial influence. The results of the water quality index revealed that the water samples from Ossiomo river is not safe for human consumption using the water quality index rating of analyzed samples according to Singh and Hussian (2016) and Rofhiwa *et al*. (2021).

Table 3 showed results of the heterotrophic bacterial counts ranged from 2.4±1.1 – 17.8±4.9 x 10^3 cfu/ml across the sampling locations and periods. Midstream sample had the highest bacterial counts of 15.9±7.0 – 17.8±4.9 x 10^3cfu/ml followed by downstream sample 8.2±6.3 – 13.6±2.4 x 10^3cfu/ml while the least count of 7.4±1.7 – 10.7±1.0 x 10^3cfu/ml) was recorded in upstream sample during the peak of rainy season (July – September). The significant difference ($P<0.05$) between midstream and upstream high bacterial count observed could be linked to increased bacteria activities in the water associated with high levels of water pollution from industrial waste and nutrient run-offs from agricultural lands. Table 4 showed results of the coliform counts were upstream samples had the least values ranged from 23 – 126 MPN/100 ml, followed by downstream sample 34 – 152 MPN/100 ml while midstream sample had the highest values of 76 – 201 MPN/100 ml. The high bacterial load observed in this study may be due to the impact of anthropogenic activities and other forms of contaminants into this surface water. These findings were in accordance with the reports of Adieze *et al.* (2016), who reported that high counts of bacterial load is a reflection of the levels of water contamination with organic matters present in the water bodies. Table 5 revealed the cultural, morphological, biochemical and physiological characteristics of bacterial isolates

The results shown in Table 6 revealed the percentage frequencies of occurrence of bacterial isolates in Ossiomo river with *Escherichia coli* 19(24) was reported to be the most prevalence isolate while *Shigella sonnei* 11.2(14.2) had the least frequency of occurrence. The values of enteric organisms recorded in this study exceeded the regulatory standards for drinking purposes, which is expected to be zero (WHO, 2011).

Table 7 showed the results of antibiogram assay of bacteria isolates. *Escherichia coli* exhibited high level of susceptibility to gentamicin (100 %) and ciprofloxacin (100 %), followed by ceftriaxone (75 %), ofloxacin (75 %) and levofloxacin (75 %) while azithromycin (50 %) and ceftazidine (50 %) were found to be resistance. But augmentin (0 %) and nitrofurantoin (0 %) showed no resistance. *Pseudomonas aeruginosa* showed high susceptibility to ceftriaxone (100 %) and ciprofloxacin (100 %), followed by pefloxacin (95 %), cefuroxime (65 %, ofloxacin (62 %) and levofloxacin (57 %) while azithromycin (50 %) and ceftazidine (50 %) were found to be resistance. Augmentin (0 %) and nitofurantoin (0%) showed no resistance. In *Klebsiella pneumonia*, cefuroxime (70 %), levofloxacin (68 %), pefloxacin (67 %), ceftazidine (62 %), ciprofloxacin (61 %) and ofloxacin (60 %) were susceptible while azithromycin (50 %) showed high resistance, ceftriaxon (0 %), gentamicin (0 %), augmentin (0 %) and nitrofurantoin (0 %) showed no resistance. *Shigella sonnie* showed higher susceptibility to pefloxacin (100 %), ceftriaxone (100 %), ciprofloxacin (100 %) and ceftazidine (100 %), followed by cefuroxin (93 %), levofloxacin (85 %), gentamicin (65 %), azithromycin (65 %) and ofloxacin (65 %). Augmentin (0 %) and nitrofurantoin (0 %) showed no resistance. *Salmonella enterica* is susceptible ceftriaxon (100 %) and ciprofloxacin (100 %), followed by cefuroxime (94 %),

levofloxacin (87 %), azithromycin (80 %), ofloxacin (76 %) and ceftaxidine (60 %) while pefloxacin (50 %) was found to be resistance. Gentamicin (0 %), augmentin (0 %) and nitrofurantoin (0 %) showed no resistance. *Proteus mirabilis* showed higher susceptibility to ciprofloxacin (100 %), ceftazidine (100 %), and cefuroxime (100 %), followed by ceftriaxone (89 %), levofloxacin (75 %), azithromycin (70 %), pefloxacin (60 %) and gentamicin (59 %) while nitrofurantoin (50 %) was found to resistance but augmentin (0 %) showed no resistance.

Conclusion: These findings showed that the Water Quality Index of Ossiomo River from the sampling locations namely upstream, midstream and downstream of the river is poor. Ossiomo River contaminated with *E. coli* and other Enterobacteriaceae is not suitable for irrigation, recreation and domestic uses. Bacterial isolates especially of the Enterobacteriaceae family are considered as good water indicators for assessment purposes of microbiological risks to humans and other aquatic lives. Hence, for surface water to retain and regain its fitness to support both human and aquatic lives, adequate management practice that foster proper sanitation surveillance which encompasses the design and implementation of surface water sanitation projects including frequent bacteriological assessment and subsequent disinfection of water source should be setup and conducted regularly.

Table 1A: Relative weight of chemical parameters

Chemical parameters[a]	Indian $standard^b$	Weight (wi)	Relative weight (wi) $Wi = \frac{wi}{\sum_{i=1} wi}$
pH	6.5-8.5	4	0.1379
Ec	300	4	0.1379
Temp	25	2	0.0690
Tds	500-2000	4	0.1379
HCO_3	244-1000	3	0.1034
Na	100	3	0.1034
Ca	75-200	2	0.0690
Mg	30-100	2	0.0690
Fe	0.3-1.0	4	0.1379
Zn	5-15	1	0.0345
		$\sum wi = 29$	$\sum$ wi = 1.000

Source (BIS, 2012)

[a]Chemical parameters in mg/L, [b]Lower value indicates desirable limit, and higher value indicates permissible limit.

Table 1B: Water quality rating based on Water Quality Index (WQI)

WQI Level	**Water Quality Status**	**Grading**
<50	Excellent water quality	A
50 – 100	Good water quality	B
100 – 200	Poor water quality	C
200 – 300	Very poor water quality	D
>300	Unsuitable for drinking purpose	E

Source: (Singh & Hussain, 2016)

Table 2: Water Quality Index of Ossiomo River within the Period of Study

Parameters	Desirable limit	Weight of each parameter (wi)	Relative weight (Wi)	Upstream	Midstream	Downstream	Mean
pH	6.5-8.5	4	0.1379	8.0	8.0	8.0	8.0
Ec	300	4	0.1379	4.43	4.40	4.37	4.40
Temp	25	2	0.0690	112.4	112.8	112.4	112.5
TDS	500-2000	4	0.1379	1.24	1.22	1.22	1.23
HCO_3	244-1000	3	0.1034	14.4	19.8	13.7	16.0
Na	100	3	0.1034	0.017	0.014	0.011	0.014
Ca	75-200	2	0.0690	0.265	0.230	0.153	0.216
Mg	30-100	2	0.0690	0.444	0.447	0.238	0.376
Fe	0.3-1.0	4	0.1379	130	245	146	173.7
Zn	5-15	1	0.0345	5.18	2.12	5.36	4.22
		$\sum wi = 29$	$\sum wi = 1.00$	$\sum WQI = 275.75$	$\sum WQI = 394.01$	$\sum WQI = 291.45$	$\sum WQI = 320.39$

Key: Ec- Electric conductivity, Temp- Temperature, TDS- Total Dissolve Solid, HCO_3- Bicarbonate, Na- Sodium, Ca- Calcium, Mg- Magnessium, Fe- Iron, Zn- Zinc

Table 3: Total Heterotrophic Bacterial Counts Isolated from Ossiomo River

Sampling stations	**July**	**Aug.**	**Sept.**	**Oct.**	**Nov.**	**Dec.**
			(x10^3±SD cfu/ml)			
Upstream	7.4±1.7	10.7±1.0	9.1±1.7	2.4±1.1	3.2±0.2	4.5±3.1
Midstream	15.9±7.0	17.8±4.9	16.0±1.8	9.2±6.7	5.4±2.1	4.7±2.4
Downstream	8.2±6.3	13.6±2.4	12.4±2.6	6.8±4.3	3.6±1.2	4.7±2.8

Values expressed as mean triplicates, World Health Organization Permissible Limits for portable= 0

Table 4: Mean Total Coliform Counts Isolated from Ossiomo River

Sampling Locations	**July**	**Aug.**	**Sept.**	**Oct.**	**Nov.**	**Dec**
Upstream	53	75	126	65	23	27
Midstream	83	201	189	121	76	79
Downstream	65	97	152	78	34	35

World Health Organization permissible limits for portable water = 0 MPN/100 ml

Table 5: Cultural, morphological, biochemical and physiological characteristics of bacterial isolates

Characteristics	**B1**	**B2**	**B3**	**B4**	**B5**	**B6**
Cell morphology	Rod	Rod	Rod	Rod	Rod	Rod
Cell arrangement	Cluster	Single	Single	Single	Single	Single
Gram reaction	Negative	Negative	Negative	Negative	Negative	Negative
Motility	+	+	-	+	-	+
Test for enzymes						
Catalase production	-	+	+	+	+	+
Spore formation	+	-	-	-	-	-
Oxidase	-	+	-	-	-	-
Coagulase	-	-	-	+	+	-
Citrate utilization	-	+	+	+	-	+
Indole	+	-	-	-	-	-
Nitrate reduction	+	-	-	+	+	+
Urease	-	-	+	-	-	+
Acid test	-	-	+	+	+	+
Sugar fermentation						
Lactose	+	+	+	-	-	-
Glucose	+	-	+	+	+	+
Galatose	+	-	+	+	+	-
Maltose	+	+	+	+	+	-
Mannitol	-	-	+	+	+	-
Identity	*Escherichia coli*	*Pseudomonas aeruginosa*	*Klebsiella pneumoniae*	*Samonella enterica*	*Shigella sonnei*	*Proteus mirabilis*

Table 6: Percentage frequency of occurrence of bacterial isolates of water samples at different sampling locations in Ossiomo River

Bacterial Isolates	**July**	**Aug**	**Sept**	**Oct.**	**Nov.**	**Dec.**	**Mean**
Klebsiella pneumonia	12(16.7)	20(16)	17(15.5)	14(20)	7(14.6)	6(12.8)	13(16.5)
Pseudomonas aeruginosa	10(13.9)	16(12.8)	12(10.9)	7(10)	6(12.5)	17(36.2)	11.3(14.3)
Shigella sonnei	8(11.1)	18(14.4)	12(14.6)	16(22.9)	5(10.4)	4(8.5)	11.2(14.2)
Eschericha coli	15(20.8)	32(25.6)	37(33.6)	14(20)	7(14.6)	6(12.8)	19(24)
Proteus mirabilis	13(18.1)	18(14.4)	17(15.5)	10(14.8)	8(16.7)	5(10.6)	12(15)
Salmonella enterica	14(19.4)	21(16.8)	11(10)	9(12.9)	15(31.3)	9(19.2)	13(17)
Total	**72**	**125**	**110**	**70**	**48**	**47**	**79**

Table 7: Antibiotic susceptibility pattern of bacterial isolates

Bacterial	No. of Isolate	LEV 5	PEF 5	CRO 30	GEN 10	CIP 5	CXM 30	AZM 15	OFL 5	CAZ 30	AUG 30	NIT 300 (µg)
P. mirabilis	2	2(75)	1(60)	2(89)	2(59)	2(100)	1(10)	1(70)	2(70)	1(100)	0(0)	1(50)
E. coli	2	2 (73)	1 (50)	2 (75)	2(100)	2(100)	1(50)	1(50)	2 (75)	1(50)	0(0)	0(0)
S. sonnie	2	2 (85)	1(100)	2(100)	2(65)	2(100)	1(93)	1 (65)	2 (65)	1(100)	0 (0)	0 (0)
P. aeruginosa	2	1(57)	2 (95)	2(100)	2(100)	1(100)	1(65)	1(50)	2 (62)	1(50)	0(0)	0(0)
K. pneumonia	2	2 (68)	1(67)	0 (0)	0 (0)	2 (61)	2(70)	1 (50)	2 (60)	1(62)	0(0)	0(0)
S. enterica	1	2(87)	1(50)	2(50)	0(0)	2(100)	1(94)	1(80)	2(76)	1(60)	0(0)	0(0)

Key: *Salmonella enterica, Escherichia coli, Pseudomonas aeroginosa, Klebsiella pneumonia, Proteus mirabilis, Shigella sonnie,*
LEV=Levofloxacin, PEF=Pefloxacin, CRO=Ceftriaxon, GEN=Gentamicin, CIP=Ciprofloxacin, CXM=Cefuroxime, OFL=Ofloxacin, CAZ=Ceftazidine, AUG=Augmentin,NIT=Nitrofurantoin and AZM= Azythromycin. Susceptibilty= 91-100 %, Intermediate=51-90 %, Resistance=1-50 %.

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