

## Survey And Antibiogram of *Escherichia coli* O157:H7 In Drinking Water Sources In Argungu Zone Of Kebbi State, Nigeria

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### ABSTRACT

Water Source is essential to human life, both for drinking, bathing and other domestic activities, which lead to discriminate demand and production of portable water in Kebbi State. Coliform such as *E. coli* O157:H7 is pathogenic serotype that is known to cause diarrhea leading to fluid loss and other complication like haemolytic Uremic syndrome. A gram-negative bacteria of the family Enterobacteriaceae, their presence in water signifies faecal contamination. A survey and antibiogram of *Escherichia coli* O157:H7 in drinking water sources was investigated in Argungu zone of Kebbi State, Nigeria. One hundred water samples that include those from Borehole, (40) Well, (50) Tap (8) and Dam (2) were sampled for the study. The samples were inoculated onto MacConkey agar and pinkish colonies on the media were subculture on to Eosin Methylene Blue (EMB) agar. The presumptive *E. coli* isolates that appeared as green metallic sheen on EMB were further conformed biochemically. The people within the area do not consider water source as possible health hazards associated with such water. Therefore, wells stand the risk of contamination either from human beings or other animals as severally reported in the literature. The presence of Enterohaemorrhagic *E. coli* (EHEC) O157:H7 in Argungu town indicate the need for more intense public health awareness by sanitary health officers and to also investigate water sources periodically for their bacteriological quality.

**Keywords:** *E. coli* O157:H7, Microbiological water quality, Antibiogram, Bacteria, culture

### 1.0 INTRODUCTION

*Escherichia coli* O157:H7 is one of the most commonly isolated and reported pathogen in medical laboratories that are classified into three major pathotypes: commensal strains, intestinal pathogenic (diarrheagenic) strains, and extraintestinal pathogenic strains. Water is essential for life, unfortunately, it serve as a major vehicle for disseminating infection and perhaps antibiotic resistant pathogens (WHO 2024, EPA, 2024). Atikson *et al.* (2012) observed that most of the water sources in congested urban cities of the developing countries were uncovered and often dug too close to refuse dumps, pit latrines or gutters and are often used for drinking and other demotic activities. In rural areas, there was less care for sanitary maintenance with regard to the vicinity of the ground water such as wells (Bello *et al.* 2013).

Similarly, in most cases, containers for drawing water are of tin, plastic cans, leather or rubber which were mostly old and kept very dirty and unprotected. In the study area, taps were common sight in many of the visited area. However, the taps were mostly dry and even when running the water delivered to public, the water are often turbid with objectionable standard for drinking (Abdelrahman *et al.* 2011). The study is aimed at determination and antibiogram of *Escherichia coli*

O157:H7 in drinking water sources in Argungu zone of Kebbi State, Nigeria.

### 1.1 Study Area

The study was carried out in the towns of Argungu, **Kebbi state Nigeria** which comprised the towns of Taggi, Kamba and Kangiwa, it also has Dam lying to the North part of the town. Kebbi State is located in the north-western part of Nigeria between latitude 10°N and 13°15'N and longitude 3°3'E and 6°E. It shares an extensive international border with Niger republic to the north and Benin republic to the west. Internally it is bordered to Sokoto and Zamfara States north-east and Niger state to the south. The state has a total land mass of 36,299 square kilometers.

### 2.0 MATERIALS AND METHODS

**2.1 Study Design and Sampling:** Cross sectional study was carried out using Random sampling in which 100 water samples were collected from four sampling frames of the Argungu zone. The sampling units (number of samples collected) include water from Boreholes (40), Tap (8), Wells (50) and Dams (2). The samples were collected in a 250mls bottle and transported on packed box to Microbiology laboratory, faculty of veterinary medicine for analysis.

## 2.1 Determination of Physicochemical Parameters

### 2.2.1 Temperature

Temperature of the Drinking water sources were determined in field by inserting a Mercury-in-glass thermometer (N2-Field England made Brannon Model) into the sample until when the mercury level become constant then the temperature of the water were recorded Atikson *et al.* (2012).

### 2.2.2 Determination of pH

The pH were measured using a pH meter. The pH meter was calibrated before taking pH measurements. For calibration standard buffers of pH 4.00, 7.00 and 10.00 are used. P<sup>H</sup> of water indicates the hydrogen ion concentration in water. The concept of pH was put forward by Sorenson in 1909. It is expressed as the logarithm of the reciprocal of the hydrogen ion concentration in moles/litre at a given temperature. While the alkalinity or acidity measures the total resistance to the pH change or buffering capacity, the pH gives the hydrogen ion activity. The pH scale extends from 0 (very acidic) to 14 (very alkaline) with 7 corresponding to exact neutrality at 25°C (Peter *et al.*, 2020).

### 2.2.3 Determination of Turbidity

The magnitude of the suspended solids in the sample were assessed visually by comparing clarity with that of distilled water and simply categorized as clear (C), lightly turbid (LT) or highly turbid (HT) as demonstrated by Okoro (1986).

### 2.2.4 Electrical Conductivity:

Electrical conductivity (EC) is a measure of how conductive the water is to electrical current. The greater the ion concentration, greater is the EC. Generally, higher the EC, higher is the total dissolved solids. Electrical Conductivity is an indirect measure for finding the total dissolved solids in a water body. To convert the electrical conductivity of a water sample (micro Siemens per cm,  $\mu\text{S/cm}$ ) to the concentration of total dissolved solids (ppm), the conductivity must be multiplied by a factor

between 0.46 and 0.9 (depending on the unique mixture of the dissolved materials). A widely accepted conversion factor is 0.67. TDS (ppm) = Conductivity  $\{(\mu\text{S/cm}) \times 0.67\}$ . The instrument used for measuring conductivity is conductivity meter. Bello *et al.* (2013).

## 2.3 Isolation, Identification and Antibiogram

*Escherichia coli* O157: H7 from water sources in Argungu zone, Kebbi State were isolated using standard cultural techniques followed by enumeration of aerobic mesophilic bacteria on the basis of MPN//100mL and identification using phenotypic and biochemical characterizations according to the procedures described by Aliyu *et al.*, (2018), Peter *et al* (2020), Bello *et al.*, (2013) and Cheesbrough (2006). Antibiotic profiling of isolates were carried out using disc diffusion technique according to Kirby Bearer, and interpreted according to Clinical Laboratory Standard Institute (CLSI 2017, 2018 and 2020) standards. The technique is in three stages:

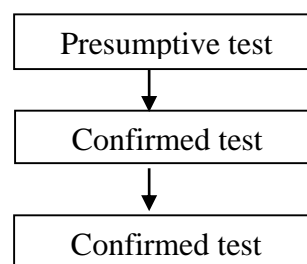


Figure 1: Stages for Identification of *Escherichia coli* O157:H7

## 2.4 Confirmation of the isolates using Molecular Analysis

DNA Extraction and Polymere Chain Reaction (PCR) These were carried out using Qiagen DNA extraction Kit as describe by Fagan, (1999). Multiplex PCR amplifications were carried out using Qiagen Hot Start<sup>®</sup> master mix and primers specific for *E. coli* STX genes (Table 1). The following components (Table 2) were mixed together on ice to make PCR cocktail prior to PCR Thermo cycling using protocol as shown in Table 3.

Table 1: Information of the primers used in the study

Oligonucleotides sequence (5'-3')	Specificity
F-CAACACTGGATGATCTCAG	Shiga
R-CCCCCTCAACTGCTAATA	toxintype 1
F-ATCAGTCGTCACACTGGT	Shiga
R-CTGCTGCTGTCACAGTGACAAA	toxintype 2

**Table 2:** Polymerase chain reaction cocktail

Cocktail constituents	Volume used
Qiagen mastermix	12.5µl
Forward primer	1.00 µl
Reverse primer	1.00 µl
DNA template	5.00 µl
Nuclease free water	5.50 µl
<b>Total Volume</b>	<b>25.0 µl</b>

**Table 3:** The PCR Protocols showing temperature and timing

S/N	PCR Step	Temperature	time
1	Initial denaturation	94°C	180 seconds
2	Denaturation	94°C	30 seconds
3	Annealing	55°C	30 seconds
4	Extension	78°C	60 seconds
5	Final extension	78°C	300 seconds

Agarose gel electrophoresis were used to identify the positive pathotype and the size of amplicons were determine by comparing with 100-bp DNA ladder (Master cycler personal Eppendorf Germany (Russo *et al* 2003).

### 3.0 RESULTS

Physicochemical properties of drinking water sources from Argungu zone in Kebbi State were carried out; the parameters studies were Temperature, pH, Biological Oxygen Demand, Dissolve Oxygen and Electric conductivity. Range values obtained for Temperature were 26°C - 28°C with an average value of 27°C while pH of all the samples ranged from 6.0 to 7.06. These suggest that the temperature of drinking water sources in State is generally ambient. Dissolved oxygen had 7.01 as the least and 8.2 as the highest value obtained and Biological Oxygen Demand value was 0.75 as least (Table 2, 3, 4 and 5).

**Table 2: Physicochemical Properties of Borehole Water from Argungu in Kebbi State**

Sampling Area	No of samples collected	Physicochemical Properties					
		PH	Temp <sup>o</sup> c	DO mgL <sup>-1</sup>	BOD mgL <sup>-1</sup>	Turbidity mgL <sup>-1</sup>	ECµS/cm
Argungu Town	16	6.53	26.56	12.09	3.07	0.92	12
		0.44±1	0.81±4	7.36±2	2.46±2	0.50±5	105.24±9
Taggi	4	7.07	26.50	7.34	1.77	0.67	158.10
		0.22±2	0.57±7	0.46±5	0.05±0	0.18±2	107.58±5
Kamba	4	6.85	26.00	7.27	1.50	0.68	196.50
		0.50±7	0.81±6	0.48±6	0.42±4	0.19±1	47.95±5
Kangiwa	16	6.59	26.18	10.14	2.74	0.78	17
		0.4±33	0.4±03	6.2±05	2.19±8	0.4±53	94.67±5

**Table 3: Physicochemical Properties of Well Water from Argungu in Kebbi State**

Sampling frame	No of samples collected	Physicochemical Properties					ECµS/cm
		pH	Temp OC	DO mgL <sup>-1</sup>	BOD mgL <sup>-1</sup>	Turbidity mgL <sup>-1</sup>	
Argungu Town	15	6.5	25.9	10.5	2.88	0.84	14
Taggi	10	6.8	26	8	1.6	0.82	17
Kamba	10	6.5	26.5	11.2	3.13	0.85	15
Kangiwa	15	6.2	26	7	1.6	0.68	18

**Table 4: Physicochemical Properties of Dam Water from Argungu in Kebbi State**

Sampling frame	No of samples collected	pH	Temp °C	DO mgL <sup>-1</sup>	BOD mgL <sup>-1</sup>	Turbidity mgL <sup>-1</sup>	ECµS/cm
Argungu Town	1	6	26	7.15	1.5	0.42	21
	0	0	0	0	0	0	0
Taggi							
Kamba	0	0	0	0	0	0	0
Kangiwa	1	7	26	7.22	1.8	0.45	25

**Table 4: Physicochemical Properties of Tap Water from Argungu in Kebbi State**

Sampling frame	No of samples collected	pH	Temp °C	DO mgL <sup>-1</sup>	BOD mgL <sup>-1</sup>	Turbidity mgL <sup>-1</sup>	ECµS/cm
Argungu Town	1	7	26	7.6	1.8	8.4	9.4
	0	0	0	0	0	0	0
Taggi							
Kamba	0	0	0	0	0	0	0
Kangiwa	0	0	0	0	0	0	0

The results of the mean bacterial aerobic count of drinking water samples from Argungu zone were presented in table 6. The mean count of Borehole obtain for all the area ranged from 1 – 25 (6.41±29) for Taggi, 41.30±6 for Kamba and 1.06±00 for Kangiwa, while Tap had 1 – 3 7.01±50 for Taggi, other area were not tested. Dam sample for Taggi had (6.69±75) and Kamba (37.83±3), well water had (6.74±20) for Taggi, (3.84±41) for Kamba and (1.10±00) for Kangiwa while the total coliform cfu/100ml mean count for all the area of Argungu zone ranged from 1 – 25 for Borehole Taggi and (26.07±13), Kamba (55.05±2) and Kangiwa 1.80±00 and (1 – 3) for Tap, Taggi 26.20±00 other area were not tested. Dam water samples had (1 – 8) 25.95±87 for Taggi and 61.97±3 for Kamba, other area were not tested. Well water had (1 – 31) (26.31±43) for Taggi, (67.68±3) for Kamba and (2.00±00) for Kangiwa. The results presented in Table 5.

**Table 5: Average Mean Bacteria count MBC (cfu/ml) of drinking water samples from Argungu Zone**

Sampling Location	Sources			
	BH	TP	DM	WW
Argungu Town	24 (6.28±2)	44 (4.22±00)	39 (1.00±00)	21
Taggi	22 (2.00±02)	26 (2.24±2)	28 (1.20±00)	24.3 (6.87±18)
Kamba	24 (1.28±00)	18 (1.34±06)	31 (2.00±00)	28 (3.65±1)
Kangiwa	22 (1.02±00)	18 (1.14±06)	20 (2.14±4)	21.2 (3.54±4)

Antibiotic susceptibility profile of the bacteria isolated from drinking water sources of the state were presented in Table 6. Thirteen (13) isolates from Argungu emirate were subjected to antibiotic susceptibility testing using nine (9) different antibiotic panels. The results obtained indicated an overall clearance zone of 32mm against Ciprofloxacin (cpx) followed by 24mm Sulphamethoxazole (sup), 20mm against Erythromycin (Ery), oxytetracyclin 18mm Neomycin (14mm), Streptomycin 10mm and Gentamycin (8mm). All the 13 isolates proved resistant to Ampicillin and Tetracyclin.

**Table 6: Results of Antibiotic Susceptibly Profiling of E. coli: O157:H7 in Drinking Water Sources of Argungu Emirate In Kebbi State**

Sampling Type	AMP	ERY	GEN	CPX	STR	TET	OXY	SULP	NEO
	00	20	8	30	10	4	18	24	14
	00	20	8	30	10	4	18	24	14
Borehole Water	00	20	8	32	10	4	18	24	14
	00	20	8	30	10	4	18	24	14
	00	20	8	30	10	4	18	24	14
Well water	00	18	8	36	10	4	18	24	16
	00	18	8	36	10	4	18	24	16

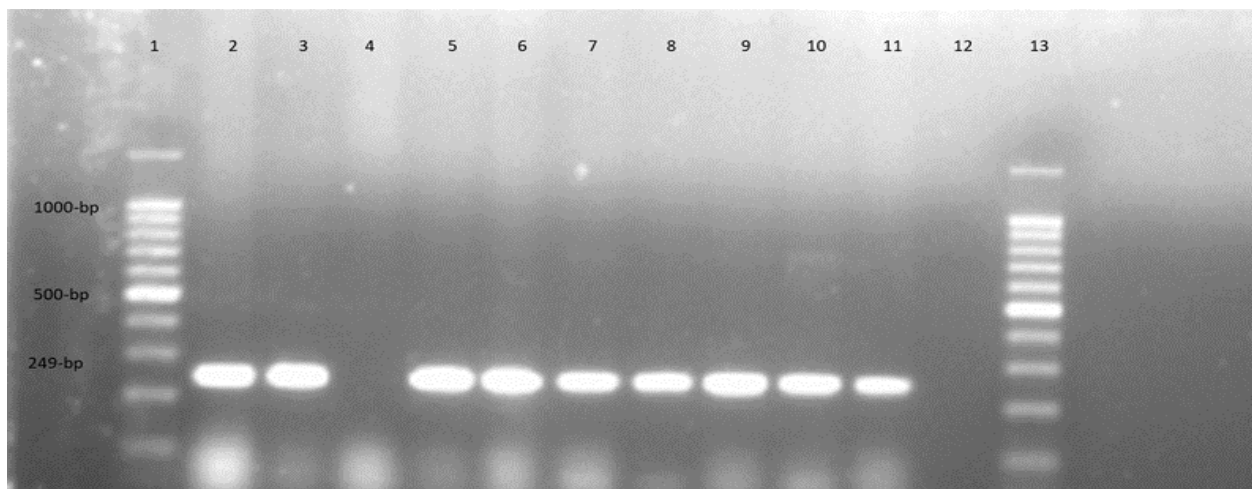
00	18	8	36	10	4	18	24	16
00	18	8	36	10	4	18	24	16
00	18	8	36	10	4	18	24	16
00	18	8	36	10	4	18	24	16

**Keys:** AMP – Ampicillin, ERY – Erythromycin, GEN – Gentamycin, CPX – Ciprofloxacin, STR – Streptomycin, TET - Tetracycline, OXY – Oxytetracycline, SULP – Sulphamethoxazole, NEO - Neomycin

Representative of the identified isolates were subjected to Polymerase Chain Reaction with the aim of amplifying the *stx*<sub>1</sub> and *stx*<sub>2</sub> and H7 genes of *E. coli* O157:H7. Nine (9) out of thirteen isolates possessed *stx*<sub>2</sub> gene while *stx*<sub>1</sub> gene was present in only one out of the thirteen isolates and had eight (8) H7 gene.

**Table 7: Molecular studies to confirm the *E. coli* O157:H7 isolates**

Sources	No. of Sample screened	No. of sample positive for <i>stx</i> <sub>1</sub>	No. of sample positive for <i>stx</i> <sub>2</sub>	No. of samples positive for H7
Borehole	40(4)	0	2	2
Tap	8(1)	1	1	0
Well Water	50(6)	0	3	3
Dam	2(2)	0	3	3



**Plate 4.1: Gel image of the amplicon of the shiga-toxin I and II gene DNA extracted from the *E. coli* O157:H7 isolates in the study 1% Agarose gel of shiga toxin I and II gene DNA extracted from the *E. Coli* O157:H7. Isolates from samples of different sources**

**Keys:**

Lane 1= 100bp ladder

Lane 2= kamba (Borehole)

Lane 3= Argungu Town (Tap water)

Lane 4= Kamba (Tap Water)

Lane 5= Taggi (Borehole)

Lane 6= Kangiwa (Borehole)

Lane 7= Argungu Town (Well water)

Lane 8= Taggi (Dam)

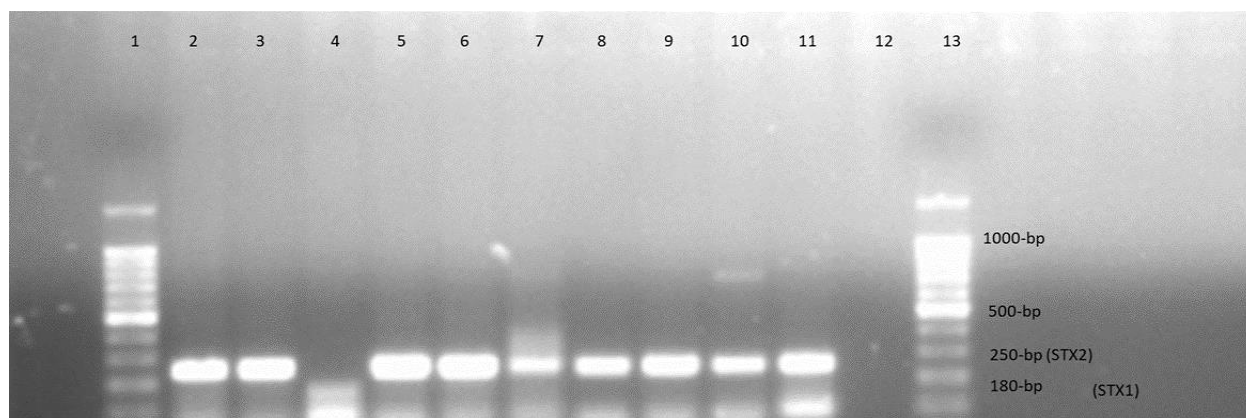
Lane 9= Kamba

Lane 10= Kangiwa (Borehole)

Lane 11= Kangiwa (Tap Water)

Lane 12= Negative Control

Lane 13= DNA Marker



**Plate 4.2: Agarose Gel image of shiga-toxin I and II gene DNA extracted from the *E. coli* O157:H7 isolates from samples of different sources**

**Key:**

Lane 1=	100bp ladder
Lane 2=	kamba (Borehole and well water) (stX <sub>2</sub> )
Lane 3=	Argungu Town (Tap water) (stX <sub>1</sub> ) (stX <sub>2</sub> )
Lane 4=	Kamba (Borehole) (stX <sub>2</sub> )
Lane 5=	Taggi (Borehole) (stX <sub>2</sub> )
Lane 6=	Kangiwa (Well water) (stX <sub>2</sub> )
Lane 7=	Argungu (Well water) (stX <sub>2</sub> )
Lane 8=	Taggi (well water) (stX <sub>2</sub> )
Lane 9=	Kamba (Borehole) (stX <sub>2</sub> )
Lane 10=	Kangiwa (Well water) (stX <sub>2</sub> )
Lane 11=	Kangiwa (Well water) (stX <sub>2</sub> )
Lane 12=	Negative Control
Lane 13=	DNA Marker

#### 4.0 DISCUSSION

Areas in Argungu zone of Kebbi State studied were found to use water from Tap, Borehole, Well and Dam as main sources of drinking water, The results obtained from the Study indicated that out of (100) hundred samples of drinking water tested, thirteen samples were contaminated with *Escherichia coli* O157:H7. It is worthy to note that all the units of water in the zone had one or more culture and Biochemical test positive isolate;

The results highlighted that the few samples that were contaminated may be due to poor hygienic environment while the remaining (87) samples that are not contaminated may be due to good practice of processing and adequate treatment of the water supply, this is in agreement with the results of Bello *et al.* (2013) and peter (2020)

Physicochemical properties of all the water sources determined includes Temperature, Biological Oxygen demand, Dissolve oxygen, pH and electric conductivity.

Temperature values in the study are ranged from 26-28.1<sup>0</sup>C, with an average value of 27.7<sup>0</sup>C. These suggest that the temperature are generally ambient and high temperature negatively impact water quality by enhancing the growth of microorganisms. It affects biological, chemical and physical activities in the water as reported by (Yilmaz and Koc, 2014 & Yahaya *et al.* 2021, Abdelrahman *et al.* 2019, Adeyemi *et al.* 2021).

However, pH of 6.0 to 7.06 recorded for the study area is consistent with results obtained by Abdulrahman *et al.* (2014) and World Health Organization (2024) that recommends a pH value of 6.5 or higher for drinking water to prevent corrosion. Electric conductivity values of the drinking water were 8.23 as least and as the higher value 12.22  $\mu\text{Scm}^{-2}$ . It gives an indication of the amount of total dissolved substitution in water as reported by Yilmaz *et al.* (2014).BOD values ranged from 0.75 to 2.1mgL<sup>-1</sup>, as the least value and the highest value were 2.1mgL<sup>-1</sup>

On the other part, the image of PCR showed nine (9) out thirteen samples poses *stx<sub>2</sub>* gene while *stx<sub>1</sub>* gene was present in only one of the thirteen isolates from Argungu zone, while eight (8) H7 gene were observed, this is similar to what Yusuf *et al.* (2018) obtained in Sokoto state.

## 5.0 CONCLUSION

Argungu zone was found to depend mostly on wells water followed by boreholes as their main sources of drinking water, surprisingly some communities are still using raw Dam water as their drinking water source. All of the drinking water sources studied in Argungu zone can be regarded as good based on their physicochemical properties, although slight pH adjustment may be required in some instances. Some of these sources, including well water, are not portable due to objectionable contamination with *E. coli* O157:H7 many of which are resistant to some commonly used antibiotics.

Similarly, ciprofloxacin, gentamycin, Erythromycin, Streptomycin, sulphamethoxazole, Neomycin and oxytetracycline are still potent on the isolates. PCR was employed to ascertain the nature of genes involved in the pathogenicity or otherwise of the isolates.

PCR was employed to ascertain the nature of genes involved in the pathogenicity or otherwise of the isolates. This study has provided information on the genetic profiling of *E. coli* O517: H7 in drinking water sources of Argungu zone in Kebbi state.

The findings of this work could also point to measures to be adopted for effective water disinfection strategy to supply portable water to the residents or communities so that water borne diseases could be prevented.

It is recommended that the pH of water from boreholes and taps should be adjusted before consumption. However, availing separate water sources for animal and human consumption through fencing the water sources in order to prevent animals from contaminating water for human consumption would improve the quality of the drinking water Sources in the area Authorities should strengthen efforts to provide portable drinking water by regular disinfection, bacteriological examination and regular inspection of open drinking water sources in the area of the state. Furthermore, studies focusing on the prevailing antibiotic resistance genes of *E. coli* O157:H7 should be conducted in order to broaden the outcome of this study.

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