International Journal of Pathogen Research

10(4): 18-30, 2022; Article no.IJPR.89495
ISSN: 2582-3876

Occurrence, Physicochemical Properties and Antibiotic Resistance Patterns of Enteric Bacteria Isolated from Well Water in Ilara-Mokin, Ondo State, Nigeria

Funmilayo Olanike Alajo [a,b*] and Felix Akinsola Akinyosoye [b]

[a] Department of Microbiology, Elizade University, P.M.B. 002, Ilara-Mokin, Nigeria.
[b] Department of Microbiology, School of Life Sciences, Federal University of Technology, P.M.B. 704, Akure, Nigeria.

Authors' contributions

This work was carried out in collaboration between both authors. Author FAA designed the study. Author FOA managed the literature searches, conducted the experiment, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors FAA and FOA managed the analyses of the study. Both authors read and approved the final manuscript.

Article Information

DOI: 10.9734/IJPR/2022/v10i430257

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/89495

Original Research Article

Received 12 May 2022
Accepted 18 July 2022
Published 29 August 2022

ABSTRACT

Aims: TheoCcurrence and antibiotic resistance patterns of enteric bacteria isolated from well water sources and their physicochemical profile was ascertained in this study. Molecular analysis of the bacterial isolates from well water samples was conducted via polymerase chain reaction (PCR)-based identification.
Study Design: Experimental design.
Methodology: A total of 12 wells were collected in triplicates from groundwater sources (wells), and mean values were obtained. The first batch of samples were collected during the dry season in January and February, 2020. The second batch of samples were collected during the rainy season in July 2020. The detection of coliforms and other enteric bacteria were conducted via the most probable number (MPN) method. Polymerase chain reaction (PCR)-based identification and characterization of bacterial isolates were employed. Antibiotic susceptibility test was done using the Kirby Bauer disc diffusion technique. The determination of the physicochemical properties of the well water samples were conducted following customary protocol.

**Corresponding author: Email: Olanike.alajo@elizadeuniversity.edu.ng;*

Results: Well water sample 7 had the highest count at 250 bacterial counts/100ml in the dry season and 200 bacterial counts/100ml in the rainy season, while well water sample 4, 10, and 11 had 14 bacterial counts/100ml in dry season, well water sample 2 had 140 bacterial counts/100ml in the dry season and 250 bacterial counts/100ml in the rainy season, whilst sample 11 had 7 bacterial counts/100ml in the rainy season. *Klebsiella pnuemoniae*, *Escherichia coli*, *Salmonella typhi* and *Shigella dysentariae* were the Gram-negative organisms enumerated and molecularly-identified as *Klebsiella pnuemoniae* subsp*. Pnuemoniae* HS11286, *Escherichia coli* 0157:H7, *Salmonella enterica* subsp. *Enterica* serovar *typhimurium* and *Shigella dysentariae* HNCMB 20080 from the well water samples with percentage identity of 97.19%, 87.44%, 80.45% and 96.19% respectively. *Klebsiella pnuemoniae* showed high resistant rate in augmentin at 67% in dry season and 100% in the rainy season. *Shigella dysentariae* showed 8.3% resistance to augmentin in the dry season and 75% in the rainy season. *E. coli* showed 67% and 58% resistance in the dry and rainy season respectively. Water hardness had the highest mean of 98.5 and 99.5 mg/L in dry and rainy season respectively while turbidity, and alkalinity had the least mean value at 1.2 (NTU) and 0.62 mg/L, 1.4 (NTU) and 0.7mg/L in both dry and rainy seasons.
Conclusion: Findings provided information on the poor quality of the well water, which will be useful in groundwater treatment strategies and policy formulation by appropriate groundwater source protection bodies.

Keywords: Enteric bacteria; well-water; physicochemical profile; dry season; rainy season.

1. INTRODUCTION

Water is one of the most indispensable requirements in life. It is a substance that is well valued and appreciated. For human consumption and good health, water must be free from pathogenic organisms, poisonous minerals and organic substances [1]. Most human population in semi-urban and rural areas in Nigeria rely mostly on well water as the main source of water supply for domestic use and drinking, due to inadequate provision of potable pipe borne water [2]. Groundwater such as wells can easily be fecally contaminated and thus, increase the incidence and outbreaks of water borne diseases [2]. Up to 80% of all illnesses and diseases in the world are implication of inadequate sanitation, polluted water or non-availability of water [3]. The infectious diseases caused by drinking unsafe water are the leading cause of mortality and morbidity for children under the age of 5 years and about 1.5 million deathsoCcur annually in developing countries [4]. Water is vital both as a solvent in which many of the body's solutes dissolve and as an essential part of many metabolic processes within the body [5].

There are greater chances of water-borne diseases with increase turbidity of water. This is because contaminants like viruses and bacteria can be attached to these suspended solids which protected them from disinfection by chlorination or ultraviolet (UV) sterilization [6]. The inhibited rays of sunlight by suspended particles can influence the rate of photosynthetic activity and thus reduce the dissolved oxygen level of the water [7]. Dissolved oxygen level in water bodies can be influenced because suspended particles absorb sunlight and increase the temperature of the water which consequently reduces the oxygen level of such water. Total dissolved solids are a general indicator of overall water quality. It is a measure of inorganic and organic materials dissolved in water. Increased total dissolved solids (TDS) may impart a bad odour or taste to drinking water, as well as cause scaling of pipes and corrosion [8]. Water hardness is the traditional measure of the capacity of water to react with soap [9]. The WHO permissible level for total hardness is 500 mg/l as $CaCO_3$. Water hardness can be classified as soft, moderate soft, slightly soft, slightly hard, moderate hard and excessively hard [10].

Groundwater pollutionoCcurs when pollutants are released to the ground and make their way down into groundwater by erosion or leaching [11]. The presence of enteric pathogens in drinking water sources is of great concern, therefore, legislation either in Europe, United States and other countries requires analysis of indicators to determine the microbiological quality of these water sources. The analysis of several microorganisms classed as either indicator or index organisms are the most useful tool to determine the potential presence of pathogens in waters [12]. Hence, theoCcurrence and antibiotic resistance patterns of enteric bacteria isolated from well water in Ilara-Mokin metropolis was ascertained in this study. This study set out to

isolate and characterize molecularly the enteric bacteria isolated from selected well water sources in Ilara-Mokin, Ondo State, carry out sensitivity tests on the isolate, determine the resistance of the isolates and estimate the physicochemical properties such as pH, conductivity, hardness, alkalinity, acidity, total dissolved solids, temperature and turbidity of the well water.

2. MATERIALS AND METHODS

2.1 Study Area Description

The study took place in Ilara-Mokin, which is located in the central part of Ifedore local government in Ondo State, Nigeria. Ilara-Mokin is an urban settlement, well known for her rich and preserved culture, academics, agriculture and tourism. It is about 12 km from Akure, the state capital of Ondo State [13].

2.1.1 Sample sites

Well water samples were collected from 12 (twelve) selected compounds in Ilara-mokin town, Ifedore Local Government, Ondo State, Nigeria. Assessments of the selected compounds were done by using a well-planned questionnaire and their addresses were noted.

2.2 Sample Collection

A total of 12 wells were used for this study. Samples were collected in triplicate from each well, and mean values were obtained. The first batch of samples were collected during the dry season in January and February, 2020. The second batch of samples were collected during the rainy season in July 2020. The well water samples were aseptically collected from selected areas in Ilara-Mokin, Ifedore, Nigeria, using sterile well fetcher and seven hundred and fifty milliliters (750 ml) polypropylene sample bottles. The samples were collected according to internationally recommended methodology. Samples were kept at 4oC until arrival to laboratory.

2.3 The Most Probable Number Analysis of Well Water Samples

The MacConkey broth (Hi-Media, India), was prepared in single and double strength in test tubes with Durham's tubes in inverted position and was autoclaved. Three sets of test tubes containing five tubes in each set; one set with 10ml double strength (DS) other two sets containing ten milliliters of single strength (SS). Using sterile pipettes, 10ml of the well water was transferred into each of Double Strength broth tubes. One milliliter of the well water sample was transferred into one set of single

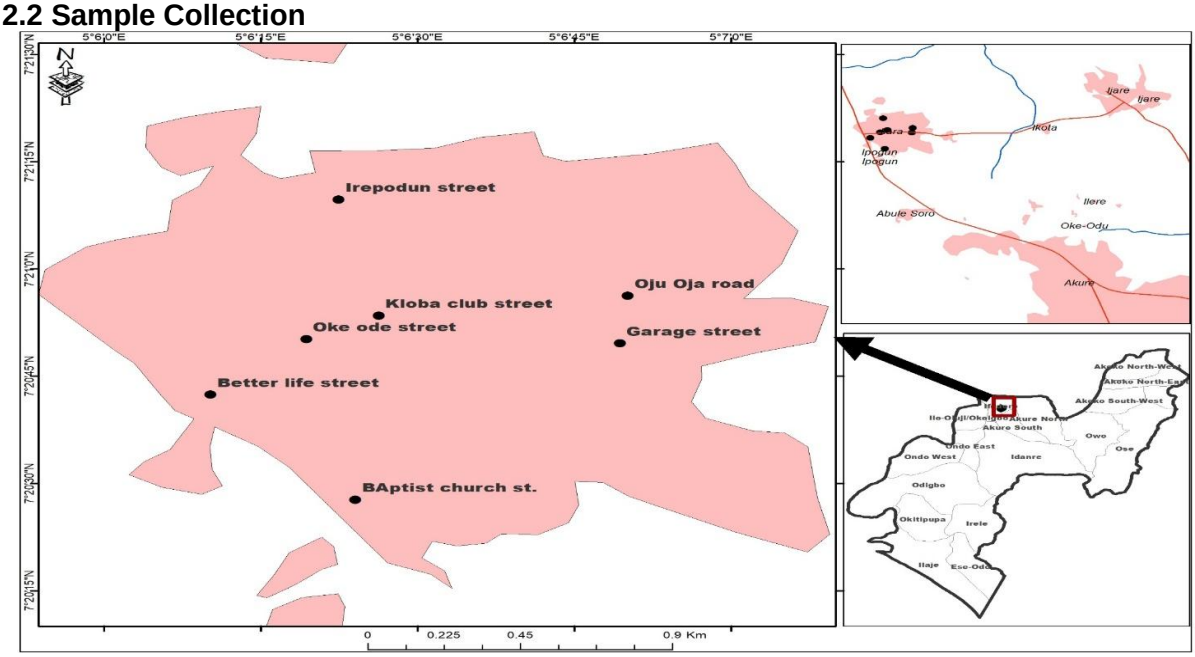

Fig. 1. Locality map of Ilara-Mokin, Nigeria showing sample collection locations

strength broth and 0.1ml well water sample was transferred into the last set of Single Strength broth tubes. The tubes were incubated at 37oC for 24 hours, after which they were observed for gas production in Durham's tube and for colour change of the media. Number of positive results from each set was recorded and compared with the standard chart to give presumptive bacterial counts per 100 ml of well water sample as described by Sagar, [14].

To ascertain the presence coliforms, confirmatory test was done as some bacteria can produce acid and gas from lactose fermentation. A loop full of suspension from a positive tube was inoculated into 3 ml lactose broth and brilliant green lactose fermentation tube and the inoculated lactose broth fermentation tubes was incubated at 37oC and were inspected for gas formation after 24 hours and 48 hours.

A loop full of the suspension from a positive tube was inoculated on to selective media agar plates; Eosin Methylene Blue (EMB) agar (Hi-Media, India), Simion Citrate agar (Hi-Media, India), and Salmonella-Shigella agar (Hi-Media, India). The plates were incubated at 37oC for 24 h, after which colonies were examined macroscopically. Pure isolates were achieved by sub-culturing on nutrient agar plates as described by Sagar [14].

2.4 Enumeration and Identification of Bacterial Isolates

Serial dilution and standard pour plate method using one (1) ml of 10^{-4} diluent of the well water samples was employed as bacterial colonies were enumerated on nutrient agar (Hi-Media, India) and MacConkey agar (Hi-Media, India) plates as demonstrated by Fawole and Osho, [15]. The bacteria petri-plates were incubated for duration of 18-24 hours at 37ºC. The bacterial colonies were then sub-cultured for identification through molecular analysis. Biochemical confirmatory tests were conducted on the isolated bacteria including; Gram reaction, motility, urease, indole, citrate utilization and sugar fermentation tests for their identification and compared with Bergey's manual of systematic bacteriology [16].

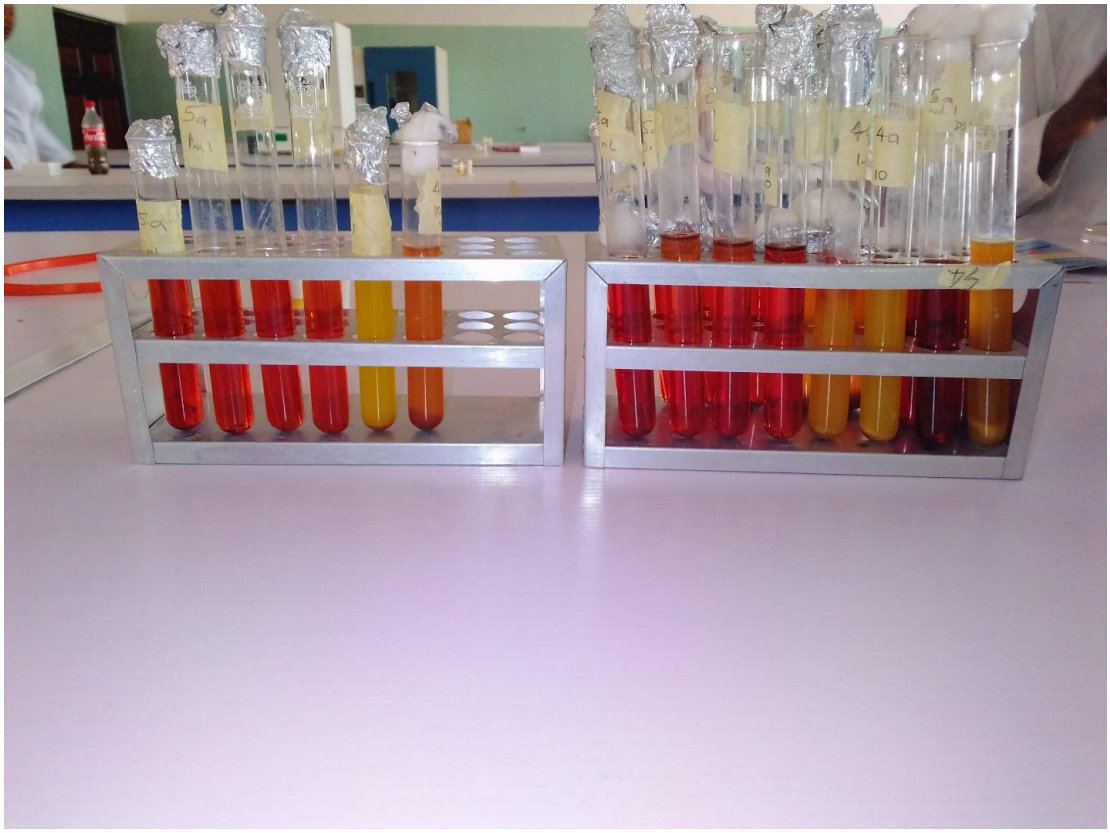

Plate 1. Positive tubes showing positive results in selective media inoculated with bacterial strains

2.5 Molecular Analysis of Bacterial Isolates from Well Water Samples

Polymerase chain reaction (PCR)-based identification and characterization of bacterial isolates were carried out with automated DNA extraction of bacterial isolates according to the technique described by Gurakan et al. [17]. The 16SrRNA gene of the bacteria was amplified using the primer pair 27F-5′-AGAGTTTGATCCTGGCTCAG-3', and 1492R 5'GGTTACCTTGTTACGACTT-3'. PCR extension was carried as described by Sambrook et al. [18]; the PCR profile used was at initial denaturation temperature of 94 ˚C for 3 mins, followed by 30 cycles of 94 ˚C for 60 sec, 56 ˚C for 60 sec, 72 ˚C for 120 seconds and the final extension temperature of 72 ˚C for 5 minutes followed by sequencing and genetic make-up blasting using the National center for biotechnological information (NCBI) server carried out by the Bioscience unit of the International Institute for Tropical Agriculture (IITA), Ibadan, Nigeria. PCR-sequencing was conducted using DNA Sanger sequencing and data was analyzed by ABI Sequencing Analysis software (version 5.2).

2.6 Antibiotics Sensitivity Test

Antibiotic susceptibility test was carried out on all the identified isolates from the twelve wells sampled, during the dry season and during the rainy season, using standard disc diffusion techniques as recommended by Clinical Laboratory Standard Institute (CLSI) [19]. Ten (10) antibiotics of different classes were tested. The antibiotics and their concentration were as follow; gentamycin (30 µg), streptomycin, (30 µg), augmentin (10 µg), ciprofloxacin (30 µg), sparfloxacin (10 µg), pefloxacin (30 µg) ofloxacin (10 µg), amoxicillin (30 µg), co-trimoxazole (septrin) (30 µg), chloramphenicol (30 µg). The antibiotics were chosen based on their importance in treating human infections caused by gram negative bacteria such *as Esherichia coli, Salmonella typhi, Shigella dysentariae and Klebsiella pnuemoniae.* Standardized inoculum was prepared by making a direct broth suspension of discrete colony selected from 18 – 24 hours culture using sterile peptone water. The suspension was adjusted to match the 0.5 McFarland for study standard as prepared by Bayode et al. [20] with trivial amendments. The dried surface of Nutrient agar plate was inoculated by pouring the standardized inoculums on the plate and then rotated for even distributing on the plate. Excess inoculum was removed by decanting it into a bowl containing disinfectant liquid. The surface of the inoculated plates was allowed to dry before applying the drug-impregnated disk. The antibiotic discs were dispensed aseptically onto the surface of the inoculated agar plate. Each disc was pressed down to ensure complete contact with the agar surface. The plates were inverted and incubated at 35oC for 18 – 24hours and diameter of zone of inhibition was read and recorded. The results of the susceptibility testing were interpreted using standard interpretative charts of CLSI [19].

2.7 Determination of the Physico-chemical Properties of the Well Water Samples

The physicochemical properties of the water samples comprising pH, electrical conductivity (mS/cm), and total dissolved solids (mg/l) of the water samples were determined using a multi-parameter analyzer (HI98194, PH/ORP/EC/DO), temperature (˚C) was determined using mercury-in-glass thermometer (ACCU-SAFE Thomas Scientific, New Jersey, US). Alkalinity (mg/l), and acidity (mg/l) was determined using titration method, turbidity (NTU) was determined via spectrophotometer (Benchtop Spectro-photometer S6003, China). These physical and chemical properties were selected in order to evaluate their influence on the microbial indicators and enteric bacteria in the water sample.

3. RESULTS AND DISCUSSION

3.1 Total Viable Bacterial Counts during Dry Season and Rainy Season

Table 1 shows the total viable bacterial counts during dry season and rainy season. Large bacterial counts ranging from 7 to 250 bacterial counts/100ml was recorded. In the dry season, sample one had 17 bacterial counts/100 ml which increased to 39 bacterial counts/100 ml in the rainy season. Sample two had a high count of 140 bacterial counts/100 ml which increased to 250 bacterial counts/100 ml. However, sample three had a decrease from 47 bacterial counts/100 ml to 26 bacterial counts/100 ml in the rainy season. Sample six bacterial counts remained the same at 21 bacterial counts/100 ml. Sample 7 in the dry season had 250 bacterial counts/100ml but in the rainy season was reduced to 200 bacterial counts /100 ml. Sample

8 to 12 had values ranging from 7 to 20 bacterial counts/100 ml.

The high value of bacterial counts recorded ranged from 7 to 250/bacterial counts per 100ml from the wells indicated that the well water was faecally-contaminated probably as a result of agricultural surface runoff, seepage or exposure to birds and other pest faeces. This is in agreement with Tambe et al. [21] who also recorded that ground water samples were frequently contaminated with human faecal organisms. There was an increase in the total bacterial count in the rainy season, increase from 140 bacterial counts per 100 ml during the dry season, to 250 bacterial counts per 100 ml during the rainy season. This may be due to rain fall. Erosion may wash these organisms from areas where they had been deposited to the well resulting in increased contamination. *Escherichia coli* was isolated from all the well samples, while *Salmonella typhi* was isolated from 58% of the well water samples.

The detection of *E. coli* is an indication of faecal organisms present in the water samples in low and high concentration. The high concentrations of *E. coli* in the groundwater samples in this study is similar to the findings of related studies such as Ghimire et al. [22]; Sharma et al. [23] as they observed high concentrations of *E. coli* in surface drinking water samples. Kirstein et al*.* [24] also reported *E. coli* values that ranged between 1 and >200 per 100 mL sample in drinking water of Sagarmatha national park, Nepal.

3.2 Ccurrence of Bacteria in Well Water Samples

Table 2 revealed the bacteria isolated from the well water samples. Four organisms were isolated, which were identified as *Escherichia coli, Klebsiella pnuemoniae, Shigella dysentariae* and *Salmonella typhi. Escherichia coli* were 100% present in all the samples in the dry season and in the rainy season. *Klebsiella pnuemoniae* and *Shigella dysentariae* were 100% present in the rainy season but 83% and 75% present respectively in the dry season. *Salmonella typhi* were the least isolated with 58% presence rate in the dry season and 50% in the rainy season.

The results of relatively high concentrations of *Salmonella* species in this study is consistent with the findings of Kovacic et al. [25] who enumerated *Salmonella* species from groundwater sources. Possible sources and reason of *Salmonella* in ground water may be attributed to inappropriately composted manure that may be transferred to fresh water via the flow paths of the soil or within the surface run-off water in line with the observation of Jacobsen and Bech [26] as the groundwater source is surrounded by arable farm lands. The detection of *Shigella dysentariae* in the well water samples indicated the presence of faecal materials present in different concentrations. The relative concentrations of *Shigella dysentariae* in this current study is in agreement with the findings reported by Rasel et al. [27], who isolated *Shigella* species from surface water. Similarly, a study conducted in Yaounde by Yongsi [28] observed that *Shigella dysentariae* had 0.24%oCcurrence from variety of drinking water. The presence of *Shigella dysentariae* in household well water could be due to poor sanitation and contamination with faecal material.

3.3 Biochemical Characteristics of Bacterial Isolates from Well Water

Klebsiella pnuemoniae, *Escherichia coli*, *Salmonella typhi* and *Shigella dysentariae* were the Gram-negative bacterial organisms enumerated from the well water samples in this study as illustrated in Table 3.

3.4 Molecular Identity of Bacterial Isolates from Well Water Samples

Klebsiella pnuemoniae subsp*. Pnuemoniae* HS11286, *Escherichia coli* 0157:H7 strain sakai DNA, *Salmonella enterica* subsp. *Enterica serovar Typhimurium* strain LT2 and *Shigella dysentariae* strain HNCMB 20080 were molecularly-identified from the well water samples with percentage identity of 97.19%, 87.44%, 80.45% and 96.19% respectively (Table 4). The molecular identity of the enteric bacteria enumerated from the well water samples in this study is in alliance with the observations of Abada et al. [29]. Recently, universal primers have been used to amplify the conserved region of 16S rRNA through the PCR technique [30]. The outcome of the molecularly-characterized enteric bacteria in this study could be attributed to the fact that most common health hazards associated with drinking water resulted from contamination by pathogenic Gram-negative bacteria as supported by Abada et al*.* [29].

Table 1. Total viable bacterial counts during dry season and rainy season

Sample Bacterial counts /100ml	Bacterial counts/100ml
	Dry season	**Rainy season**
1	17	39
2	140	250
3	47	26
4	14	20
5	35	39
6	21	21
7	250	200
8	20	17
9	35	17
10	14	12
11	14	7
12	15	12

Table 2. Ccurrence of bacteria in well water samples

Isolates	**Samples**												
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**% Occurrence**
Dry season													
Escherichia coli	+	+	+	+	+	+	+	+	+	+	+	+	100
Klebsiella pnuemoniae	+	+	-	+	+	+	+	+	+	+	+	-	83
Shigella dysentariae	+	-	+	+	+	+	+	-	+	+	+	-	75
Salmonella typhi	+	-	-	+	+	+	-	+	-	+	-	+	58
Rainy season													
Escherichia coli	+	+	+	+	+	+	+	+	+	+	+	+	100
Klebsiella pnuemoniae	+	+	+	+	+	+	+	+	+	+	+	+	100
Shigella dysentariae	+	+	+	+	+	+	+	+	+	+	+	+	100
Salmonella typhi	+	-	-	-	-	+	+	-	-	+	+	+	50

Key; - = negative, += positive

Table 3. The biochemistry of bacterial isolates from well water samples

Biochemical test	***Klebsiella pnuemoniae***	***Escherichia coli***	***Salmonella typhi***	***Shigella dysentariae***
Gram stain	-	-	-	-
Indole test	-	+	-	-
Citrate utilization test	+	-	-	-
Motility	-	+	+	-
Urease test	+	-	-	-
Glucose	+	+	+	+
Sucrose	+	+	-	-
Lactose	+	+	-	-

Key; - = negative, += positive

3.5 Physicochemical Profile of Well Water Samples

Hardness mean value obtained was 98.5 and 99.5 mg/L in dry and rainy season respectively while turbidity, mean values were 1.2 and 1.4 Nephelometric turbidity unit (NTU), acidity mean values were 1.9 and 2.0 mg/L. Alkalinity mean value were 0.6 and 0.7 mg/L respectively. Also, Total Dissolved Solids in both dry and rainy season were14.0 and 15.3mg/L (Fig. 2). The mean values of the physicochemical parameters of the well water samples were evaluated. The mean value for pH in dry and rainy season was 6.5 is within the range of standard maximum permissible limit set by WHO [31] which range from 6.0 to 8.5. A similar result was also reported by Chinedu et al. [32]. They recorded pH values between the range of 5.96 and 7.17 for surface water samples collected from Ota, Ogun State, Nigeria. The findings from this study in respect of

the pH are similar to the findings of Oyem et al. [33]. Acidic water has been identified to cause damage to cells of mucous membrane, eyes and skin irritation [34,35]. The alkalinity of water samples may be due to the presence of bicarbonates lost into the soil and percolated into the underground soil via rain water essentially in the rainy season. The temperature values of the well water samples reported in this study could be attributed to an increase in environmental temperature and imbalances the environment, may also be a factor in temperature and the increase in biological productivity [36]. A high-water temperature can cause damage to some organisms which require cold water and high oxygen. The hotter the water, the less dissolved oxygen it contains. The temperature of the water does not change at the same speed as that of the air: it cools less quickly. Indeed, water has a higher thermal capacity than that of air. The high conductivity value reported could be attributed to the dissolution of ionic heavy metals from industrial activities of heavy machines which later found their ways into groundwater via leaching of sub-soil layers [35]. The increased variation of the total dissolved solids in the well water samples may be as an indication of the presence of inorganic salts and small amount of organic matter in excess concentration which could make water objectionable and shorten the duration of hot water heaters. The concentration of hardness in the groundwater samples is as a result of metallic ions dissolved in the water as concentration of calcium carbonate, which is known to decrease the lather formation of soap and increase scale formation in hot-water heaters and low-pressure boilers at high levels.

Table 4. Molecular identification of bacterial isolates from the well water samples

Isolate Codes	Similarity%	Molecular identity	NCBI Gene bank accession number	Isolates identified
C1	97.19	*Klebsiella pnuemoniae* subsp. *Pnuemoniae* HS11286	NC-016845.1	*Klebsiella pnuemoniae*
C2	87.44	*Escherichia coli* 0157:H7 strain sakai DNA	NC-002695.2	*Esherichia coli*
C3	80.45	*Salmonella enterica subsp. Enterica serovar Typhimurium* strain LT2.	NC-003197.2	*Salmonella typhi*
C4	96.19	*Shigella dysentariae* strain HNCMB 20080	NZ-CP061527.1	*Shigella dysentariae*

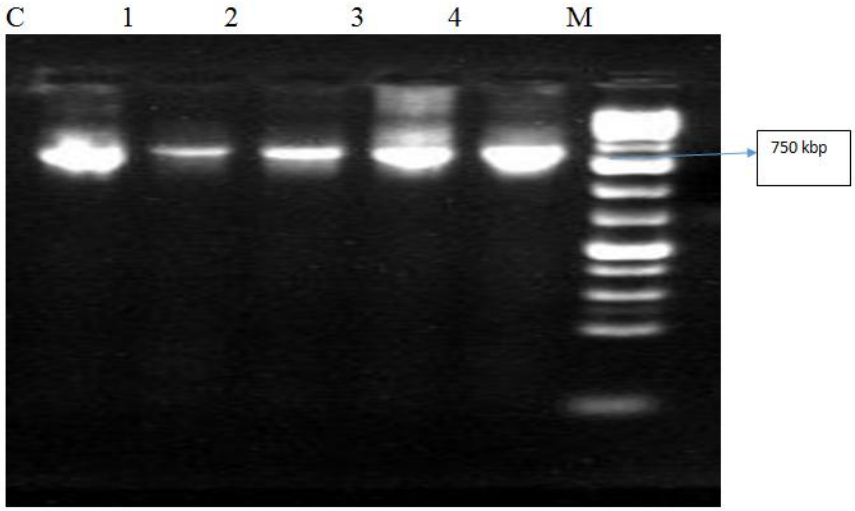

Plate 2. Polymerase Chain Reaction (PCR) of bacterial isolates on ethidium bromide electrophoretic gel

1=-Esherichia coli, 2- Klebsiella pnuemoniae, 3- Salmonella typhi, 4- Shigella dysentariae M=DNA Marker, C=control

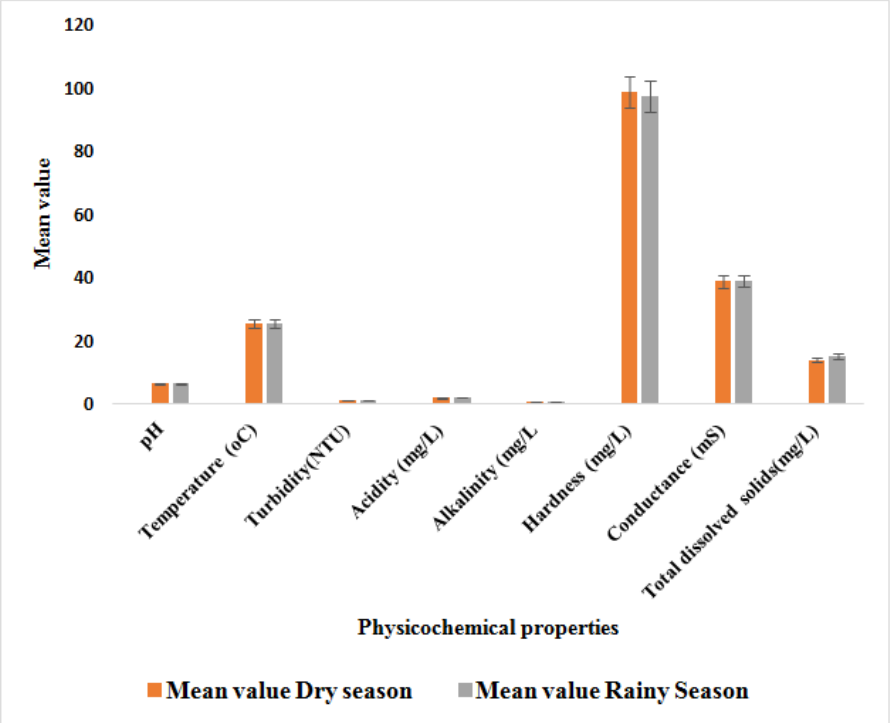

Fig. 2. Physicochemical properties of the well water samples during the dry and rainy season

3.6 Resistance Pattern of Bacterial Isolates from Well Water Samples

Fig. 3 revealed the resistance of the bacterial isolates to ten different antibiotics during the dry season. *E. coli* and *Klebsiella pnuemoniae* showed resistance of 67% to augmentin, Salmonella typhi showed resistance rate of 41% while *Shigella dysentariae* showed 8.3% resistance rate to augmentin. *Salmonella typhi* showed 50% resistance to amoxacillin, *Klebsiella pnuemoniae* showed 41% resistance rate, while *Escherichia coli* showed 18% resistance to amoxacillin. *Shigella dysentariae* showed 41% resistance to Streptomycin, while *Salmonella typhi* and *Klebsiella pneumoniae* gave 25% resistance rate. *Klebsiella pneumoniae* gave higher resistance rate to augmentin, amoxacillin, and streptomycin at 87%, 100% and 87% respectively during the rainy season as showed in Fig. 4.

The findings in the present study indicated that the enteric bacteria recovered showed high levels of resistance to antibiotics that are commonly used in treating infectious water borne related diseases in the study areas. This could contribute to the spread and persistence of antibiotic-resistant bacteria and resistance determinants in humans and the environment. *Klebsiella pnuemoniae* gave the highest resistance rate, the ability of the organism to produce the enzymes extended spectrum beta lactamases may be a contributing factor for this resistance. One of the most important factors contributing to the spread of antibiotic-resistance in bacteria has been attributed to the fact that in most developing countries including Nigeria, diarrheal diseases are treated with inadequate regimen of antimicrobials and often empirically without first identifying the pathogens. Nontongana et al. [37] had reported high resistance rate of coliforms to antibiotics.

Antibiotics used in animal husbandry as growth promoters could be another factor contributing to antibiotic-resistant bacteria in ground water sources as the gut microbial flora of these animals end up developing resistance to these antibiotics when they get to the environment, and passing the same to autochthonous bacteria in ground water. The dissemination of antibiotic resistance in the environment may have potential negative clinical implications for therapeutic advancement [38,39]. Resistance of *E. coli* from drinking water sources to commonly used antibiotics has been documented [40,41].

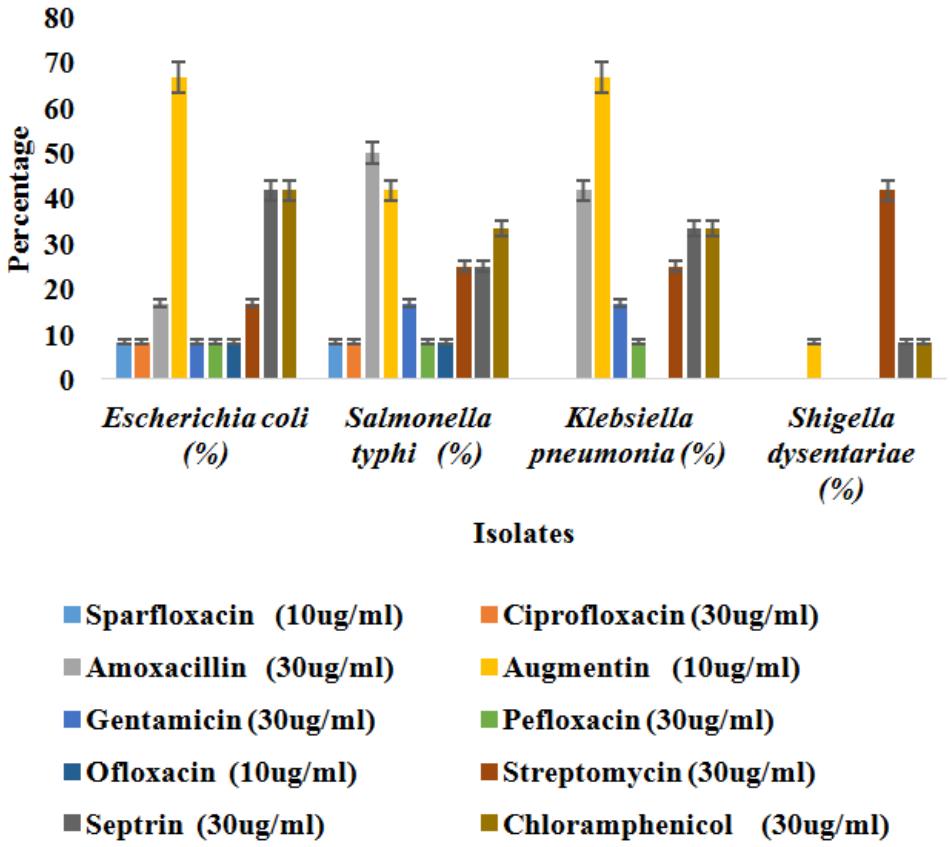

Fig. 3. Resistance of bacterial isolates from well water samples during dry season

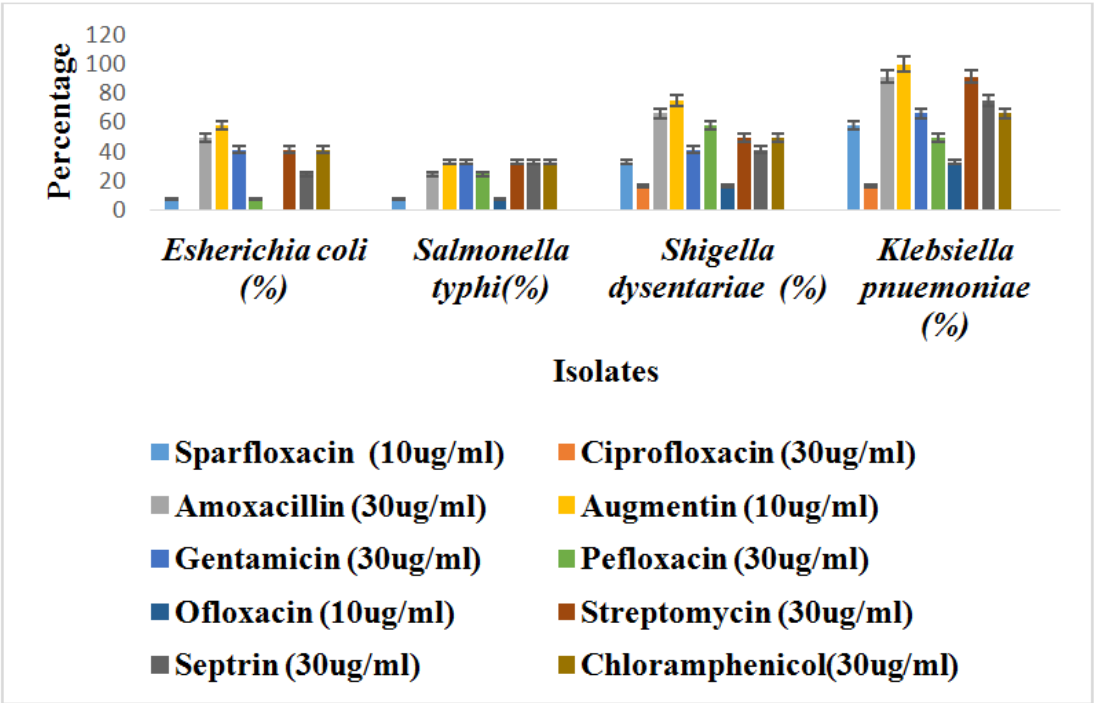

Fig. 4. Resistance of bacterial isolates from well water samples during the rainy season

4. CONCLUSION

This study provided information on the poor quality of well water in Ilara-Mokin, Ondo State, Nigeria. It also revealed the extent of contamination of well water sources. The observations from this study suggest the need for focused intervention on water source protection and sanitation practices, especially during the rainy season by appropriate authorities to conduct quality assessment of well water sources from time to time in order to ensure good quality drinking water availability. The study outcome may also be useful in the management strategies and decision making as well as planning and policy formulation by the appropriate water source protection bodies or agencies.

ACKNOWLEDGEMENTS

Authors appreciate the efforts of technical Staff of Microbiology Department, Federal University of Technology, Akure in providing a conducive environment for conducting this study.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Folorunso OR, Adetuyi FT, Laseinde EA. Onibi GE Microbiological Assessment of well water at Different Durations of Storage. Pak J Biological Sci. 2014;17(2):198-205.
2. Aboh EA, Giwa FJ, Giwa A. Microbiological assessment of well waters in Samaru, Zaria, Kaduna, State, Nigeria. Ann Afr Med. 2015;14(1):32.
3. Olowe BM, Oluyege JO, Famurewa O. Prevalence of waterborne diseases and microbial assessment of drinking water quality in Ado-Ekiti and its environs, Southwestern, Nigeria. Brit Microbiol Res J. 2016;12 (2):1-13.
4. Bahiru AA, Emire SA, Ayele AK. The prevalence of antibiotic resistant *Escherichia coli* isolates from fecal and water sources. Acad J Microbiol Res. 2013;1(1):001-010.
5. Gacesa M, Zahnle KJ. (2019, December). Escape of high-altitude water and D/H fractionation driven by non-thermal processes on Mars. In AGU Fall Meeting Abstracts. 2019;52C-05.
6. Jung S, Hemmatian T, Song E, Lee K, Seo D, Yi J, Kim J. Disinfection treatments of disposable respirators influencing the bactericidal/bacteria removal efficiency, filtration performance, and structural integrity. Polymers. 2021;13(1):45.
7. Yisa J, Tijani JO, Oyibo OM. Underground water assessment using water quality index. repository.futminna.edu.ng; 2012.
8. Napacho ZA, Manyele SV. Quality assessment of drinking water in Temeke District (part II): Characterization of chemical parameters. Afr J Environ Sci Technol. 2010;4(11):775-789.
9. Soni VK, Visavadia M, Gosai C, Hussain MD, Mewada MS, Gor S, Salahuddin K. Evaluation of physico-chemical and microbial parameters on water quality of Narmada River, India. Afr J Environ Sci Technol. 2013;7(6):496-503.
10. Bernard E, Ayeni N. Physicochemical analysis of groundwater samples of Bichi local government area of Kano State of Nigeria. World Environ. 2012;2(6):116-119.
11. Ackah M, Agyemang O, Anim AK, Osei J, Bentil NO, Kpattah L, Hanson JEK. Assessment of groundwater quality for drinking and irrigation: the case study of Teiman-Oyarifa Community, Ga East Municipality, Ghana. Proceed Intl Acad Ecol Environ Sci. 2011;1(3-4):186.
12. Odonkor ST, Ampofo JK. *Escherichia coli* as an indicator of bacteriological quality of water: an overview. Microbiol res. 2013;4(1):5-11.
13. Adu BW, Amusan BO, Oke TO. Assessment of the water quality and Odonata assemblages in three waterbodies in Ilara-Mokin, south-western Nigeria. Intl J Odonatol. 2019;22(2):101-114.
14. Sagar NA, Pareek S, Sharma S, Yahia EM, Lobo MG. Fruit and vegetable waste: Bioactive compounds, their extraction, and possible utilization. Comp Rev Food Sci Food Safety. 2018;17(3):512-531.
15. Fawole MO, Oso BA. Characterization of Bacteria In: Laboratory Manual of Microbiology. Spectrum Book Ltd., Ibadan, Nigeria; 2001.
16. Don, J. B., James, T. S., noel, R. K. In: Bergey's Manual of Systematic Bacteriology (Volume 2, Parts Aâ□"C, 2nd Edition). FEMS Immunology & Medical Microbiology. 2006;46(3):476–476.

Available:https://doi.org/10.1111/j.1574695x.2005.00055.x
17. Gurakan GC, Aksoy C, Ogel ZB. Differentiation of *Salmonella typhymurium* from *Salmonella enteritidis* and other *Salmonella* serotypes using random amplified polymorphic DNA analysis. Poultry Sci. 2008;87:1068-1074.
18. Sambrook, E. F. Fritsch, T. Maniatis, Cold Spring Harbor Laboratory Press, Cold Spring Harbour 1989, $ 115. ISBN 0-87969-309-6. Biologie in Unserer Zeit, 2000;20(6):285–285.
Acvailable:https://doi.org/10.1002/biuz.19900200607
19. Clinical Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing; twenty-fourth informational supplement. CLSI document M100- S24. Wayne, PA: Clinical and Laboratory Standards Institute; 2018.
20. Bayode MT, Olalemi, AO, Oladejo BO. Multiple antibiotic resistant index and detection of qnrS and qnrB genes in bacterial consortium of urine samples from clinical settings. Euro J Biol Res. 2021;11(1):45-56. 509.
Available:http://dx.doi.org/10.5281/zenodo.4304311
21. Tambe PV, Daswani PG, Mistry NF, Ghadge AA, Antia NH. A community-based bacteriological study of quality of drinking-water and its feedback to a rural community in Western Maharashtra, India. J hlth popu Nut. 2008;26(2):139.
22. Ghimire G, Adhikari B, Pradhan M. Bacteriological analysis of water of Kathmandu Valley. Med J Shree Birendra Hosp. 2013;12(2):19-22.
23. Sharma B, Parul AK, Jain U, Yadav JK, Singh R, Mishra, R.oCcurrence of multidrug resistant *Escherichia coli* in groundwater of Brij region (Uttar Pradesh) and its public health implications. Vet world. 2017;10(3):293.
24. Kirstein NN, Klaus N, Carolyn D, Subodh S. *E. coli* and Coliform Bacteria as Indicators for Drinking Water Quality and Handling of Drinking Water in the Sagarmatha National Park, Nepal. Environ Managemt Sustain Developmt. 2017;6(2):411-428.
25. Kovac˘ic A, Huljev Z, Sušic E. Ground water as the source of an outbreak of *Salmonella enteritidis.* J Epidemiol Global Hlth. 2017;1-4.
26. Bech P. Clinical Psychometrics. Wiley Blackwell, Oxford; 2012.
Available:http://dx.doi.org/10.1002/9781118511800
27. Rasel K, Faruque SM, Kamruzzaman M, Shinji Y, Shafi AQ, et al. Isolation of *Shigella dysenteriae* Type 1 and *S. flexneri* Strains from Surface Waters in Bangladesh: Comparative Molecular Analysis of Environmental *Shigella* Isolates versus Clinical Strains. Appl Environ Microbiol. 2002 68(8):3908–3913.
28. Yongsi HB. Pathogenic microorganisms associated with childhood diarrhea in Low-and-middle income countries: Case study of Yaoundé–Cameroon. Intl J Environ Res Public Hlth. 2008;5(4):213-229.
29. Abada E, Al-Fifi Z, Al-Rajab AJ, Mahdhi M, Sharma M. Molecular identification of biological contaminants in different drinking water resources of the Jazan region, Saudi Arabia, J Water Health. 2019;17(4):622-632.
30. Amir A, Zeisel A, Zuk O, Elgart M, Stern S, Shamir O, Turnbaugh PJ, Soen Y, Shental N. High-resolution microbial community reconstruction by integrating short reads from multiple 16S r RNA regions. Nucleic Acids Res. 2013;41(22):e205.
31. WHO. World health organization global frame works for sanitation and water supply; 2009.
32. Chinedu SN, Nwinyi O, Oluwadamisi AY, Eze VN. Assessment of water quality in Canaanland, Ota, Southwest Nigeria. Agric Biol J North Amer. 2011;2(4):577-583.
33. Oyem HH, Oyem IM, Ezeweali D. Temperature, pH, electrical conductivity, total dissolved solids and chemical oxygen demand of groundwater in Boji-Boji Agbor/Owa area and immediate suburbs. Res J Environ Sci. 2014;8(8):444–450.
34. Meinhardt PL. Recognizing waterborne disease and the health effects of water contamination: A review of the challenges facing the medical community in the United States. J Water Hlth. 2006;4(1):27–34.
35. Popoola LT, Yusuff AS, Aderibigbe TA. Assessment of natural groundwater physico-chemical properties in major industrial and residential locations of Lagos metropolis. Appl Water Sci. 2019;9:191.
36. Togue FK, Kuate GLO, Oben LM. Physico-Chemical characterization of the surface water of Nkam River using the Principal Component Analysis. J Mat Environ Sci. 2017;8(6):1910-1920.

37. Nontongana N, Sibanda T, Ngwenya E, Okoh AI. Prevalence and antibiogram profiling of *Escherichia coli* pathotypes isolated from the Kat River and the Fort Beaufort Abstraction Water. Intl J Environ Res Public Hlth. 2014;11:8213-8227.
38. Samie A, Guerrant RL, Barrett L, Bessong PO, Igumbor EO, Obi CL. Prevalence of intestinal parasitic and bacterial pathogens in diarrhoeal and non-diarrhoeal human stools from Vhembe district, South Africa. J Hlth, Popu Nut. 2012;27:739–745
39. Akhter A, Imran M, Akhter F. Determination of multiple antibiotic-resistant patterns and indexing among metal tolerant beta-lactamase producing *Escherichia coli.* Afr J Microbiol Res. 2014;8(7):619- 627.
40. Mishra M, Patel AK, Behera N. Prevalence of multidrug resistant *E. coli* in the river Mahanadi of Sambalpur. Curr Res Microbiol Biotechnol. 2013;1(5):239-244.
41. Oluyege JO, Dada O, Oluyege A, Olowomofe T. Multiple antibiotic resistance index of *Escherichia coli* isolated from drinking water sources in Ado-Ekiti, Ekiti State. The Experiment. 2014;28(1):1896-1905.

Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle5.com/review-history/89495