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Molecular Identification of *Escherichia coli* and New Emerging Enteropathogen, *Escherichia fergusonii*, From Drinking Water Sources in Ado-Ekiti, Ekiti State, Nigeria

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Abstract Molecular identification of environmental isolates using 16S rRNA gene is very important when accurate and decisive bacterial identification is required. This study identified bacterial isolates (n = 9) from samples of different drinking water sources using 16S rRNA gene sequence. Bacterial 16S rRNA gene was amplified using suitable primers. The amplified 16S rRNA gene sequence was compared with the sequence in NCBI sequence database using Basic Local Alignment Search Tool (BLAST) search program. The BLAST results showed that isolates S71 was 91% similar to both *E. coli* strain S51 with accession number CP015995.1 and *E. coli* strain 268-78-1 with accession number CP014092.1, isolate S177 was 91% similar to both *E. coli* strain BACO64 with accession number KU161315.1 and *E. coli* strain BAC031 with accession number KU161312.1, S31 was 91% similar to *E. coli* strain 268-78-1 with accession number CP014092.1, isolates S89 and S34 were both 91% similar to *E. coli* strain OZK2 with accession number KT440880.1, isolate S152 was 89% similar to *E. coli* strain C-XIB with accession number KJ806504.1, isolates S33 and S35 were 91% similar to *E. fergusonii* strain CGS24 with accession number KX034239.1 while S30 was 91% similar to *E. coli* strain S51 with accession number CP015995.1. The phylogenetic tree showed two different clusters. The first cluster comprises S152, S177, S89, S71 which were identified as *E. coli* strain C-XIB, BAC064, OZK2, S51 respectively while the second cluster comprises S33, S31, S30, S34, S35 which were identified as *E. fergusonii* strain CGS24, *E. coli* strain 268-78-1, S51, OZK2, *E. fergusonii* strain CGS24 respectively. This study is believed to be the first study to provide a report which differentiates *E. fergusonii* from *E. coli* and on the incidence of *E. fergusonii* in drinking water sources in Nigeria.

Keywords Water sources, *Escherichia coli, Escherichia fergusonii*, 16S rRNA gene, BLAST, Phylogenetic tree

1. Introduction

Availability of safe drinking water is a key factor underpinning public health and development of any nation. Water sources available in Nigeria include: surface water such as lakes, streams, rivers, ponds and underground water such as springs, wells, borehole (Oluyege *et al*., 2009). There is always a link between environmental sanitation or sanitary practices of any community and the quality of water sources in that community. In Nigeria, overpopulation, increased urbanization, agricultural practices, industrialization, unhygienic practices among others has affected the quality of water sources (Olowe *et al*., 2016). Studies have revealed non-conformity of many water sources in Nigeria to World Health Organization (WHO) standard (Nwidu *et al.,* 2008; Oyedeji *et al.,* 2010; Kalpana *et al.*, 2011; Ohanu *et al*., 2012; Gideon *et al.,* 2013; Sunday and Innocent, 2014; Olowe *et al.,* 2016). Some microorganisms of faecal origin have been implicitly implicated in contamination of water sources among which include the *Escherichia* species (Abdul, 2010).

Escherichia species belong to the family Enterobacteriaceae and consists of six species which include: Escherichia coli, Escherichia fergusonii, *Escherichia hermannii, Escherichia vulneris, Escherichia blattae* and Escherichia albertii (Walk *et al*., 2009). Among these species, E. coli has been mostly studied and isolated from faecally contaminated water. Hence, it is being used as faecal indicator of choice in W.H.O. guidelines for drinking water quality (Rompre *et al.*, 2002; WHO, 2006). In addition, several countries such as Europe and U.S.A. include this organism in their regulations as the primary indicator of faecal pollution (Odonkor and Ampofo, 2013).

On the other hand, *Escherichia fergusonii* was proposed

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as a new emerging species within the genus *Escherichia* by Farmer *et al.* (1985). There are certain phenotypic differences which enabled it to be differentiated from other species within genus *Escherichia,* in particular, *E. coli* which was shown by DNA hybridization to be its closest relative (64% similarity) (Wragg *et al*., 2009). It can be differentiated from *E. coli* by being sorbitol and lactose negative but adonitol, amygdalin and cellobiose fermentation positive (Foster *et al*., 2010; Simmon *et al*., 2014). *E. fergusonii* has been isolated from a wide range of human clinical specimens, including blood, urine and faeces (Gaafar *et al*., 2015) at which time, the clinical significance of the species was not known. Occasional isolations of *E. fergusonii* from clinical samples have been reported in some countries like UK, France (Freney *et al.*, 1987). In addition, it has been isolated from animals such as pigs, sheep, cattle, goats, horses, reindeer, ostriches, turkeys chickens (Oh *et al.,* 2012; Gaafar *et al.*, 2015) and water sources (Maheux *et al*., 2014) in some countries but to the best of our knowledge, its occurrence has not been reported in Nigeria. Furthermore, Chaudhury *et al.* (1999) has demonstrated the potential for *E. fergusonii* to cause enteric disease.

Due to the close similarity between *E. coli* and *E. fergusonii,* molecular identification using 16S rRNA is very paramount. This is because the use of 16S rRNA gene sequences to study bacterial phylogeny and taxonomy has been by far the most common housekeeping genetic marker used for a number of reasons. These reasons include: its presence in almost all bacteria; often existing as a multigene family or operons; the function of the 16S rRNA gene over time has not changed, suggesting that random sequence changes are a more accurate measure of time (evolution) and the 16S rRNA gene (1,500 bp) is large enough for informatics purposes (Patel, 2001). Therefore, this study aimed to identify *E. coli* and *E. fergusonii* using 16S rRNA from water sources in Ado-Ekiti, Ekiti State, Nigeria.

2. Materials and Methods

2.1. Bacterial Culture

Nine bacterial isolates from different water sources: S30, S35, S71, S89 and S152, from well samples, S177 from stream sample, S31 and S33 from borehole samples and S34 from pipe-borne sample, which were identified culturally and biochemically as *E. coli* in a previous study (Olowe *et al.*, 2015) were used for this study.

2.2. Extraction of DNA using CTAB Method

The bacterial isolates from different water sources were grown overnight in a liquid Nutrient Broth at 37°C. isolation of genomic DNA was carried out using CTAB method as described by Akinyemi and Oyelakin (2014). DNA concentration of each of the samples was measured on spectrophotometer at 260 nm and 280 nm and their genomic purity were determined.

2.3. DNA Electrophoresis

Agarose gel electrophoresis was used to determine the quality and integrity of the DNA by size fractionation on 1.0% agarose gels. Agarose gels were prepared by dissolving and boiling 1.0 g agarose in 100 mL 0.5X TBE buffer solution. The gels were allowed to cool down to about 45°C and 10 µL of 5 mg/mL ethidium bromide was added, mixed together before pouring it into an electrophoresis chamber set with the combs inserted. After the gel has solidified, 3 µL of the DNA with 5 µL sterile distilled water and 2 µL of 6X loading dye was mixed together and loaded in the well created. Electrophoresis was done at 80 V for 2 hours. The integrity of the DNA was visualized and photographed on UV light source. The extracted genomic DNA was used as template DNA for amplification of the 16S rRNA gene.

2.4. PCR Analysis using 16S Primer

PCR analysis was run with the universal primer for bacteria. The PCR mix comprised of 1 µL of 10 X buffer, 0.4 µL of 50 mM $MgCl_2$, 0.5 µL of 2.5 mMdNTPs, 0.5 µL 5 mM forward and reverse primers, 0.05 µL of 5 units/µlTaq with 2 µL of template DNA and 5.05 µL of distilled water to make-up 10 µL reaction mix. The PCR profile used has an initial denaturation temperature of 94°C for 3 minutes, followed by 35 cycles of 94°C for 30 sec, 60°C for 40 sec, 72°C for 60 sec and the final extension temperature of 72°C for 5 minutes and the 10°C hold forever.

2.5. Purification of PCR Products

The amplicon was further purified before the sequencing, using 2 M sodium acetate wash techniques. To about 10 µL of the PCR product, 1 µL 2 M NaAct pH 5.2 was added followed by 20 µL absolute ethanol and kept at -20°C for 1 hour. It was then spun at 10,000 rpm for 10 minutes, washed with 70% ethanol and air dried. After which, was resuspended in 5 µL sterile distilled water and kept at 4°C for sequencing.

2.6. PCR for Sequencing

The primer used for the reaction was 16S. The PCR mix used included 0.5 µL of BigDye Terminator Mix, 1 µL of 5X sequencing buffer, 1 µL of M13 forward primer with 6.5 µL distilled water and 1 µL of the PCR product making a total of 10 µL. The PCR profile for Sequencing is a rapid profile, the initial Rapid thermal ramp to 96°C for 1 minute followed by 25 cycles of rapid thermal ramp to 96°C for 10 seconds, rapid thermal ramp to 50°C for 5 seconds and rapid thermal ramp to 60°C for 4 minutes, then followed by rapid thermal ramp to 4°C and hold forever.

2.7. Purification of PCR Sequencing Products

The PCR sequence product was also purified before sequencing running using 2M Sodium Acetate wash techniques. To 10 µL of the PCR product, 1 µL 2M NaAct

pH 5.2 was added, then 20 µL absolute ethanol was added and kept at -20°C for 1hour. It was then spun at 10,000 rpm for 10 minutes, washed with 70% ethanol and air-dried. After which was re-suspend in 5 µL sterile distilled water and kept at 4°C for sequencing running.

2.8. Preparation of Sample for Gene Sequencer (ABI 3130xl Machine)

The Cocktail mix is a combination of 9 µL of Hi di formamide with 1 µL of purified sequence making a total of 10 µL. The samples were loaded on the machine and data in form Adenine, Cytosine, Thyamine, and Guanine were released.

3. Results

The genomic purity for all the DNA samples was between 1.8 –2.0. An absorbance quotient value of between 1.8 and 2.0 is considered to be good and purified DNA. A ratio of less than 1.8 is indicative of protein contamination, where as a ratio of greater than 2.0 is indicative of RNA contamination. The integrity of genomic DNA samples were confirmed on 1.0% agarose gel by electrophoresis (Figure 1).

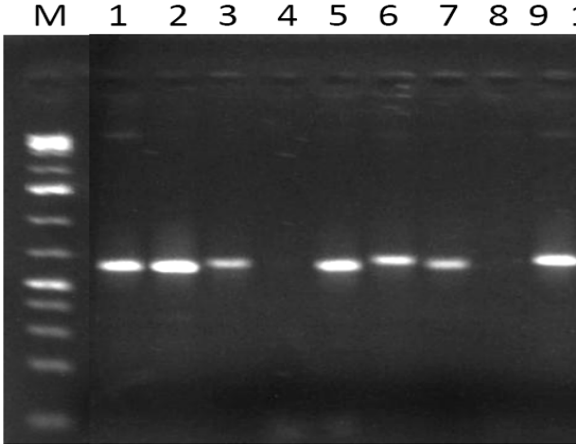


Figure 1. 1.0 % Agarose gel electrophoresis of the bacterial genomic DNA samples

The result in Figure 1 showed different band intensities. The DNA samples loaded in lane 4 and 8 had low intensity bands while other DNA samples had intense visible bands. The 16S rRNA gene of each of the bacterial isolates was amplified using suitable primer and the amplified 16S rRNA gene sequences (Figure 2-10) were then compared with the sequence in National Centre for Biotechnology Information (NCBI) gene bank database using Basic Local Alignment Search Tool (BLAST) search program. BLAST is a web based program that is able to align the search sequence to thousands of different sequences in a database and show the list of top matches. Figure 3 showed the alignment obtained from BLAST search program for the bacterial isolates. The alignment was performed by matching up each position of search sequence to each position of the sequences in the database. For each position, BLAST gave a positive score if the nucleotides matched, it also inserted gaps when performing the alignment. Each gap inserted has a negative effect on the alignment score, but with enough aligned nucleotides, the negative effect was overcome and the gap was accepted in the alignment (Figure 11). Table 1 showed the summary of the results of the sequences obtained. The BLAST result showed that isolates S71 was 91% similar to both *E. coli* strain S51 with accession number CP015995.1 and *E. coli* strain 268-78-1with accession number CP014092.1, isolate S177 was 91% similar to both *E. coli* strain BACO64 with accession number KU161315.1 and *E. coli* strain BAC031 with accession number KU161312.1, S31 was 91% similar to *E. coli* strain 268-78-1 with accession number CP014092.1, isolates S89 and S34 were both 91% similar to *E. coli* strain OZK2 with accession number KT440880.1, isolate S152 was 89% similar to *E. coli* strain C-XIB with accession number KJ806504.1, isolates S33 and S35 were 91% similar to *E. fergusonii* strain CGS24 with accession number KX034239.1 while S30 was 91% similar to *E. coli* strain S51 with accession number CP015995.1.

CGGGGGGGCTTATTCTTTAGTAAGTCGAGCGGTAGCACGAAGGAGCTTGCTTCTTTGCTGAAGAGTGGCGGACGGGTGAGTA
ATGTCTGGGAAACTGCCTGATGGAGGGGGATAACTACTGGAAACGGTAGCTAATACCGCATAACGTCGCAAGACCATTGAGG
GGGACCTTCGGGCCTCTTGCCATCGGATGTGCCCAGATGGTATTAGGTTGTTGGTGGGGTAACGGCTCACCTAGGCGACGATC
CCTAGCTGGTCTGAGAGGATGACCAGGCACACTGGAACTGAGACACGGTCCATACTCCTACGGGAGGGATCAGTGGGGAATA
TTGCACAATGGGCGCAAGCCTGATGCAGCCATGCCGCGTGTATGAAGAAGGGCTTCGGGTTGTATAGTACTTTCAGCGAGGA
GGAAGGGAGTAAAGTTAATACCTTTGCTCATTGACGTTACCCGCAGAATAAGCACCTGCTAACTCCCTGCCAGCTGCCGCGGT
AATACGGAGGGTGCAAGCGTTCTTCGTAATTACTGTGCGTAGAGGGCACGCAGGCGGTTTGTTAAGTCGGATGTGATATCCCC
GGGCTCAACCTGGGAACTGCATCTGATACTGGCAAGCTTGAGTCTCGTAGAGGGCGGTAGAATTCCGTGTGTAGCGGTGAAA
TGCATATAGATCTGGAGGAATACCGGTGGCGAAGGCTGCCCCCTGGACGAAGACTGACGCTCATGTGCGAAAGCGTGGATCA
GCATACTATGATTATATACCCTGGTAGTCCACGCCGTAAACGATGTCCACTTGGAGGTTGTGCCCTTGAGGCGCGGCTTCAGG
AGCTCACGCGTTTGTCCACCGCCTGGGGTGTACGGCCGCTAAGTTAAAACTGTATGTATTGAGTAGCTGCACCGCACAAGCGG
TGGAGTTGCTAGCTTTATTCGACGCAACGAGCTTAACCTGACCTGATCTGACATCCACGGAACTCTCCATAGATGCTCTGCGC
CGTCGAGCTACAGTGATACACGTGCTGCATGGCAGTCATCACTCGATGTGGTGAATTG

|CP015995.1|	Escherichia coli strain S51, complete genome	91%	CP015995.1
|CP014092.1|	Escherichia coli strain 268-78-1, complete genome	91%	CP014092.1

Figure 2. Complete genome sequence of PCR product of 16S rRNA gene of isolate S71 from well water sample

GTAATGTCTGGGAAACTGCCTGATGGAGGGGGATAACTACTGGAAACGGTAGCTAATACCGCATAACGTCGCAAGACCATTG
AGGGGGACCTTCGGGCCTCTTGCCATCGGATGTGCCCAGATGGTATTAGGTTGTTGGTGGGGTAACGGCTCACCTAGGCGACG
ATCCCTAGCTGGTCTGAGAGGATGACCAGGCACACTGGAACTGAGACACGGTCCATACTCCTACGGGAGGGATCAGTGGGGA
ATATTGCACAATGGGCGCAAGCCTGATGCAGCCATGCCGCGTGTATGAAGAAGGGCTTCGGGTTGTATAGTACTTTCAGCGA
GGAGGAAGGGAGTAAAGTTAATACCTTTGCTCATTGACGTTACCCGCAGAATAAGCACCTGCTAACTCCCTGCCAGCTGCCGC
GGTAATACGGAGGGTGCAAGCGTTCTTCGTAATTACTGTGCGTAGAGGGCACGCAGGCGGTTTGTTAAGTCGGATGTGATATC
CCCGGGCTCAACCTGGGAACTGCATCTGATACTGGCAAGCTTGAGTCTCGTAGAGGGCGGTAGAATTCCGTGTGTAGCGGTG
AAATGCATATAGATCTGGAGGAATACCGGTGGCGAAGGCTGCCCCCTGGACGAAGACTGACGCTCATGTGCGAAAGCGTGGA
TCAGCATACTATGATTATATACCCTGGTAGTCCACGCCGTAAACGATGTCCACTTGGAGGTTGTGCCCTTGAGGCGCGGCTTC
AGGAGCTCACGCGTTTGTCCACCGCCTGGGGTGTACGGCCGCTAAGTTAAAACTGTATGTATTGAGTAGCTGCACCGCACAAG
CGGTGGAGTTGCTAGCTTTATTCGACGCAACGAGCTTAACCTGACCTGATCTGACATCCACGGAACTCTCCATAGATGCTCTG
CGCCGTCGAGCTACAGTGATACACGTGCTGCATGGCAGTCATCACTCGATGTGGTGAATTGCCTGGATTTAGCCCGCCACGAT
GCCGCTACGCCTTCATCTGATGCCTA

|KU161315.1| Escherichia coli strain BAC064 16S ribosomal RNA gene, partial sequence 91% KU161315.1

|KU161312.1| Escherichia coli strain BAC031 16S ribosomal RNA gene, partial sequence 91% KU161312.1

Figure 3. Partial sequence of PCR product of 16S rRNA gene of isolate S177 from stream water sample

GGGGGCTTATTCTTTAGTAAGTCGAGCGGTAGCACGAAGGAGCTTGCTTCTTTGCTGAAGAGTGGCGGACGGGTGAGTAATGT
CTGGGAAACTGCCTGATGGAGGGGGATAACTACTGGAAACGGTAGCTAATACCGCATAACGTCGCAAGACCATTGAGGGGG
ACCTTCGGGCCTCTTGCCATCGGATGTGCCCAGATGGTATTAGGTTGTTGGTGGGGTAACGGCTCACCTAGGCGACGATCCCT
AGCTGGTCTGAGAGGATGACCAGGCACACTGGAACTGAGACACGGTCCATACTCCTACGGGAGGGATCAGTGGGGAATATTG
CACAATGGGCGCAAGCCTGATGCAGCCATGCCGCGTGTATGAAGAAGGGCTTCGGGTTGTATAGTACTTTCAGCGAGGAGGA
AGGGAGTAAAGTTAATACCTTTGCTCATTGACGTTACCCGCAGAATAAGCACCTGCTAACTCCCTGCCAGCTGCCGCGGTAAT
ACGGAGGGTGCAAGCGTTCTTCGTAATTACTGTGCGTAGAGGGCACGCAGGCGGTTTGTTAAGTCGGATGTGATATCCCCGGG
CTCAACCTGGGAACTGCATCTGATACTGGCAAGCTTGAGTCTCGTAGAGGGCGGTAGAATTCCGTGTGTAGCGGTGAAATGC
ATATAGATCTGGAGGAATACCGGTGGCGAAGGCTGCCCCCTGGACGAAGACTGACGCTCATGTGCGAAAGCGTGGATCAGCA
TACTATGATTATATACCCTGGTAGTCCACGCCGTAAACGATGTCCACTTGGAGGTTGTGCCCTTGAGGCGCGGCTTCAGGAGC
TCACGCGTTTGTCCACCGCCTGGGGTGTACGG

|CP014092.1| Escherichia coli strain 268-78-1, complete genome 91% CP014092.1

Figure 4. Complete genome sequence of PCR product of 16S rRNA gene of isolate S31 from borehole water sample

GAGCTTGCTTCTTTGCTGAAGAGTGGCGGACGGGTGAGTAATGTCTGGGAAACTGCCTGATGGAGGGGGATAACTACTGGAA
ACGGTAGCTAATACCGCATAACGTCGCAAGACCATTGAGGGGGACCTTCGGGCCTCTTGCCATCGGATGTGCCCAGATGGTAT
TAGGTTGTTGGTGGGGTAACGGCTCACCTAGGCGACGATCCCTAGCTGGTCTGAGAGGATGACCAGGCACACTGGAACTGAG
ACACGGTCCATACTCCTACGGGAGGGATCAGTGGGGAATATTGCACAATGGGCGCAAGCCTGATGCAGCCATGCCGCGTGTA
TGAAGAAGGGCTTCGGGTTGTATAGTACTTTCAGCGAGGAGGAAGGGAGTAAAGTTAATACCTTTGCTCATTGACGTTACCCG
CAGAATAAGCACCTGCTAACTCCCTGCCAGCTGCCGCGGTAATACGGAGGGTGCAAGCGTTCTTCGTAATTACTGTGCGTAGA
GGGCACGCAGGCGGTTTGTTAAGTCGGATGTGATATCCCCGGGCTCAACCTGGGAACTGCATCTGATACTGGCAAGCTTGAGT
CTCGTAGAGGGCGGTAGAATTCCGTGTGTAGCGGTGAAATGCATATAGATCTGGAGGAATACCGGTGGCGAAGGCTGCCCCC
TGGACGAAGACTGACGCTCATGTGCGAAAGCGTGGATCAGCATACTATGATTATATACCCTGGTAGTCCACGCCGTAAACGA
TGTCCACTTGGAGGTTGTGCCCTTGAGGCGCGGCTTCAGGAGCTCACGCGTTTGTCCACCGCCTGGGGTGTACGGCCGCTAAG
TTAAAACTGTATGTATTGAGTAGCTGCACCGCACAAGCGGTGGAGTTGCTAGCTTTATTCGACGCAACGAGCTTAACCTGACC
TGATCTGACATCCACGGAACTCTCCATAGATGCTCTGCGCCGTCGAGCTACAGTGATACACGTGCTGCATGGCAGTCATCACT
CGATGTGGTGAATTGCCTGGATTTAGCCCGCCACGATGCCGCTACGCCTTCATCTGATGCCTAGCGTCCTGCCGGTACTCAAT
GCAACTGGCAC

|KT440880.1| Escherichia coli strain OZK2 16S ribosomal RNA gene, partial sequence 91% KT440880.1

Figure 5. Partial sequence of PCR product of 16S rRNA gene of isolate S89 from well water sample

GATAACTACTGGAAACGGTAGCTAATACCGCATAACGTCGCAAGACCATTGAGGGGGACCTTCGGGCCTCTTGCCATCGGAT
GTGCCCAGATGGTATTAGGTTGTTGGTGGGGTAACGGCTCACCTAGGCGACGATCCCTAGCTGGTCTGAGAGGATGACCAGG
CACACTGGAACTGAGACACGGTCCATACTCCTACGGGAGGGATCAGTGGGGAATATTGCACAATGGGCGCAAGCCTGATGCA
GCCATGCCGCGTGTATGAAGAAGGGCTTCGGGTTGTATAGTACTTTCAGCGAGGAGGAAGGGAGTAAAGTTAATACCTTTGC
TCATTGACGTTACCCGCAGAATAAGCACCTGCTAACTCCCTGCCAGCTGCCGCGGTAATACGGAGGGTGCAAGCGTTCTTCGT
AATTACTGTGCGTAGAGGGCACGCAGGCGGTTTGTTAAGTCGGATGTGATATCCCCGGGCTCAACCTGGGAACTGCATCTGAT
ACTGGCAAGCTTGAGTCTCGTAGAGGGCGGTAGAATTCCGTGTGTAGCGGTGAAATGCATATAGATCTGGAGGAATACCGGT
GGCGAAGGCTGCCCCCTGGACGAAGACTGACGCTCATGTGCGAAAGCGTGGATCAGCATACTATGATTATATACCCTGGTAG
TCCACGCCGTAAACGATGTCCACTTGGAGGTTGTGCCCTTGAGGCGCGGCTTCAGGAGCTCACGCGTTTGTCCACCGCCTGGG
GTGTACGGCCGCTAAGTTAAAACTGTATGTATTGAGTAGCTGCACCGCACAAGCGGTGGAGTTGCTAGCTTTATTCGACGCAA
CGAGCTTAACCTGACCTGATCTGACATCCACGGAACTCTCCATAGATGCTCTGCGCCGTCGAGCTACAGTGATACACGTGCTG
CATGGCAGTCATCACTCGATGTGGTGAATTGCCTGGATTTAGCCCGCCACGATGCCGCTACGCCTTCA

|KJ806504.1| Escherichia coli strain C-X1B 16S ribosomal RNA gene, partial sequence 89% KJ806504.1

Figure 6. Partial sequence of PCR product of 16S rRNA gene of isolate S152 from well water sample

CGGGTGAGTAATGTCTGGGAAACTGCCTGATGGAGGGGGATAACTACTGGAAACGGTAGCTAATACCGCATAACGTCGCAAG
ACCATTGAGGGGGACCTTCGGGCCTCTTGCCATCGGATGTGCCCAGATGGTATTAGGTTGTTGGTGGGGTAACGGCTCACCTA
GGCGACGATCCCTAGCTGGTCTGAGAGGATGACCAGGCACACTGGAACTGAGACACGGTCCATACTCCTACGGGAGGGATCA
GTGGGGAATATTGCACAATGGGCGCAAGCCTGATGCAGCCATGCCGCGTGTATGAAGAAGGGCTTCGGGTTGTATAGTACTT
TCAGCGAGGAGGAAGGGAGTAAAGTTAATACCTTTGCTCATTGACGTTACCCGCAGAATAAGCACCTGCTAACTCCCTGCCA
GCTGCCGCGGTAATACGGAGGGTGCAAGCGTTCTTCGTAATTACTGTGCGTAGAGGGCACGCAGGCGGTTTGTTAAGTCGGAT
GTGATATCCCCGGGCTCAACCTGGGAACTGCATCTGATACTGGCAAGCTTGAGTCTCGTAGAGGGCGGTAGAATTCCGTGTGT
AGCGGTGAAATGCATATAGATCTGGAGGAATACCGGTGGCGAAGGCTGCCCCCTGGACGAAGACTGACGCTCATGTGCGAAA
GCGTGGATCAGCATACTATGATTATATACCCTGGTAGTCCACGCCGTAAACGATGTCCACTTGGAGGTTGTGCCCTTGAGGCG
CGGCTTCAGGAGCTCACGCGTTTGTCCACCGCCTGGGGTGTACGGCCGCTAAGTTAAAACTGTATGTATTGAGTAGCTGC

|KT440880.1| Escherichia coli strain OZK2 16S ribosomal RNA gene, partial sequence 91% KT440880.1

Figure 7. Partial sequence of PCR product of 16S rRNA gene of isolate S34 from pipe-borne water sample

CTTATTCTTTAGTAAGTCGAGCGGTAGCACGAAGGAGCTTGCTTCTTTGCTGAAGAGTGGCGGACGGGTGAGTAATGTCTGGG
AAACTGCCTGATGGAGGGGGATAACTACTGGAAACGGTAGCTAATACCGCATAACGTCGCAAGACCATTGAGGGGGACCTTC
GGGCCTCTTGCCATCGGATGTGCCCAGATGGTATTAGGTTGTTGGTGGGGTAACGGCTCACCTAGGCGACTCTCCCTAGCTGG
TCTGAGAGGATGACCAGGCACACTGGAACTGAGACACGGTCCATACTCCTACCGGAGGGATCAGTGGGGAATATTGCACAAT
GGGCGCAAGCCTGATGCAGCCATGCCGCGTGTATGAAGAAGGGCTTCGGGTTGTATAGTACTTTCAGCGAGGAGGAAGGGAG
TAAAGTTAATACCTTTGCTCATTGACGTTACCCGCAGAATAAGCACCTGCTAACTCCCTGCCAGCTGCCGCGGTAATACGGAG
GGTGCAAGCGTTCTTCGTAATTACTGTGCGTAGAGGGCACGCAGGCGGTTTGTCCAGTCGGATGTGATATCCCCGGGCTCAAC
CTGGGAACTGCATCTGATACTGGCAAGCTTGAGTCTCGTAGAGGGCGGTAGAATTCCGTGTGTAGCGGTGAAATGCATATAG
ATCTGGAGGAATACCGGTGGCGAAGGCTGCCCCCTGGACGAAGACTGACGCTCATGTGCGAAAGCGTGGATCAGCATACTAT
GATTATATACCCTGGTAGTCCACGCCGTAAACGATGTCCACTTGGAGGTTGTGCCCTTGAGGCGCGGCTTCAGGAGCTCACGC
GTTTGTCCACCGCCTGGGGTGTACGGCCGCTAAGTTAAAAC

|KX034239.1| Escherichia fergusonii strain CGS24 16S ribosomal RNA gene, partial sequence 91% KX034239.1

Figure 8. Partial sequence of PCR product of 16S rRNA gene of isolate S33 from borehole water sample

GGCTTATTCTTTAGTAAGTCGAGCGGTAGCACGAAGGAGCTTGCTTCTTTGCTGAAGAGTGGCGGACGGGTGAGTAATGTCTG
GGAAACTGCCTGATGGAGGGGGATAACTACTGGAAACGGTAGCTAATACCGCATAACGTCGCAAGACCATTGAGGGGGACCT
TCGGGCCTCTTGCCATCGGATGTGCCCAGATGGTATTAGGTTGTTGGTGGGGTAACGGCTCACCTAGGCGACGATCCCTAGCT
GGTCTGAGAGGATGACCAGGCACACTGGAACTGAGACACGGTCCATACTCCTACGGGAGGGATCAGTGGGGAATATTGCACA
ATGGGCGCAAGCCTGATGCAGCCATGCCGCGTGTATGAAGAAGGGCTTCGGGTTGTATAGTACTTTCAGCGAGGAGGAAGGG
AGTAAAGTTAATACCTTTGCTCATTGACGTTACCCGCAGAATAAGCACCTGCTAACTCCCTGCCAGCTGCCGCGGTAATACGG
AGGGTGCAAGCGTTCTTCGTAATTACTGTGCGTAGAGGGCACGCAGGCGGTTTGTTAAGTCGGATGTGATATCCCCGGGCTCA
ACCTGGGAACTGCATCTGATACTGGCAAGCTTGAGTCTCGTAGAGGGCGGTAGAATTCCGTGTGTAGCGGTGAAATGCATAT
AGATCTGGAGGAATACCGGTGGCGAAGGCTGCCCCCTGGACGAAGACTGACGCTCATGTGCGAAAGCGTGGATCAGCATACT
ATGATTATATACCCTGGTAGTC

|KX034239.1| Escherichia fergusonii strain CGS24 16S ribosomal RNA gene, partial sequence 91% KX034239.1

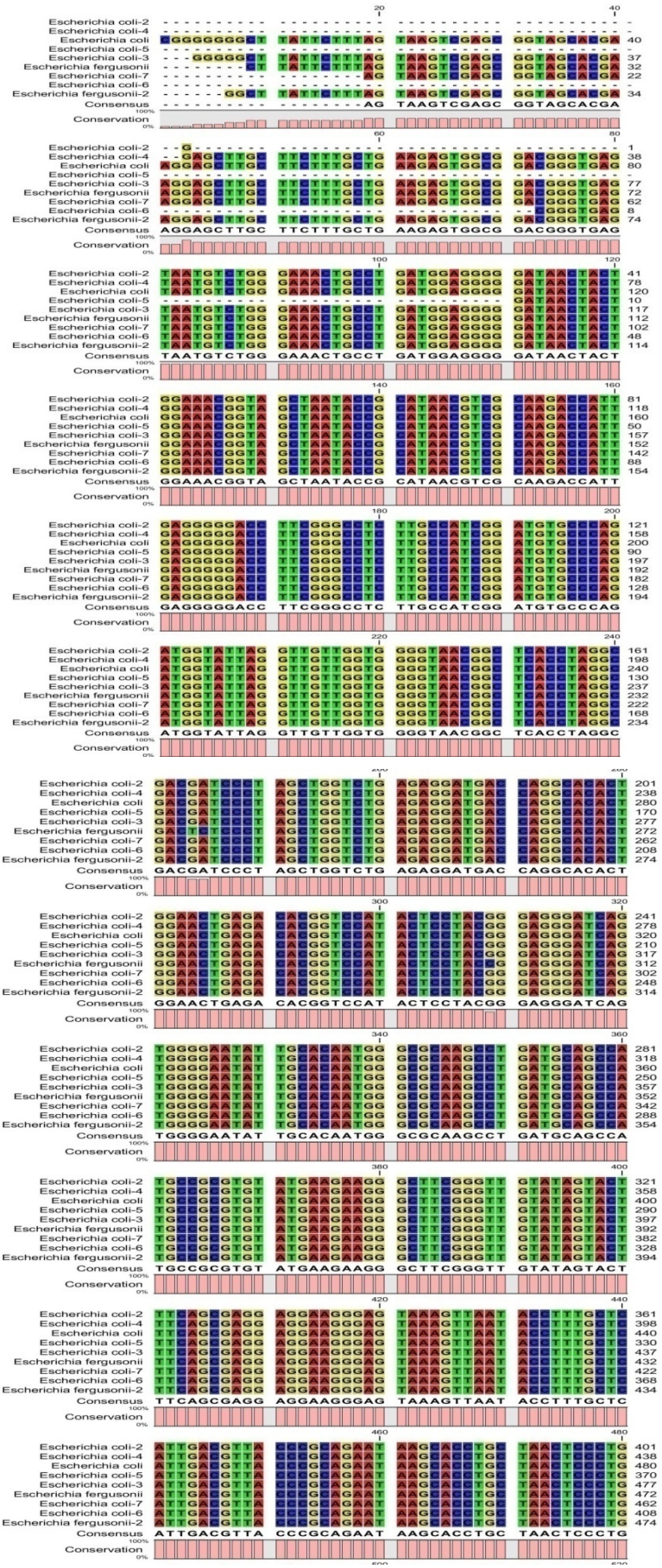
Figure 9. Partial sequence of PCR product of 16S rRNA gene of isolate S35 from well water sample

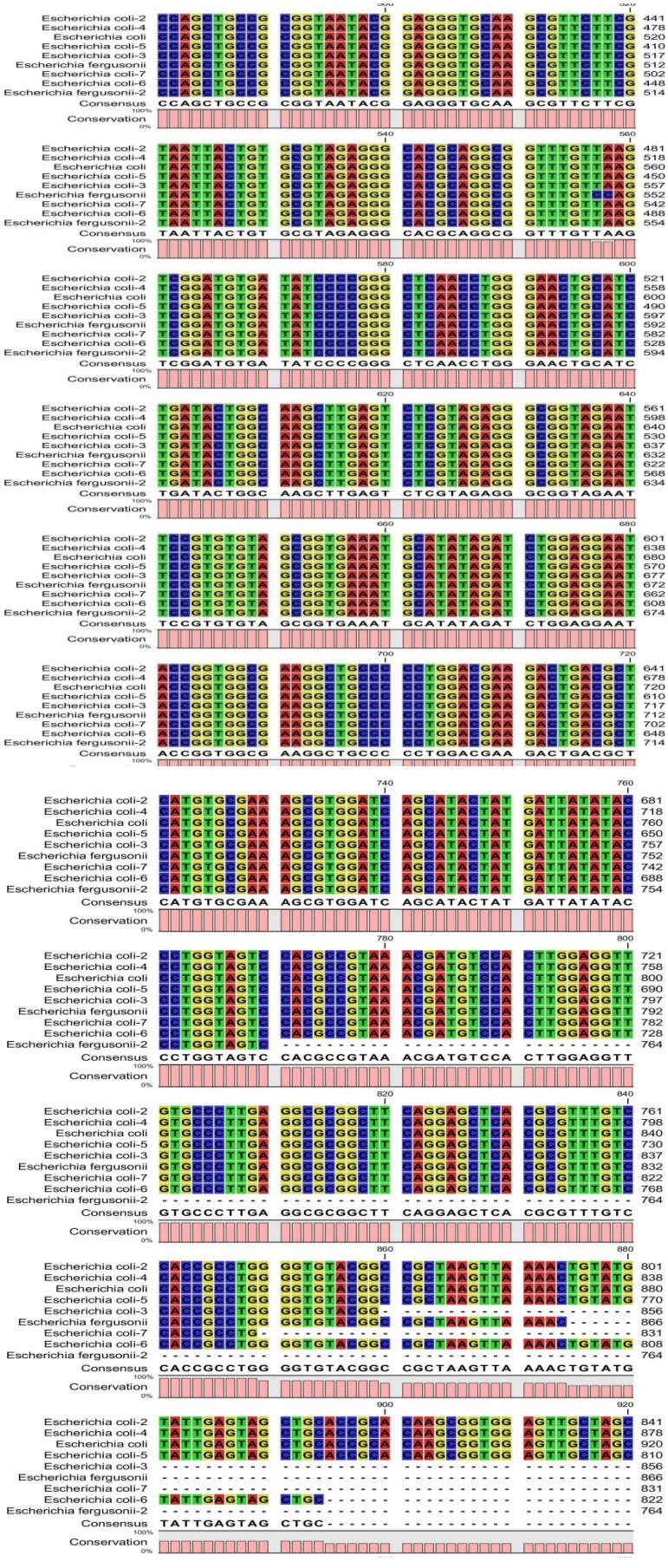
AGTAAGTCGAGCGGTAGCACGAAGGAGCTTGCTTCTTTGCTGAAGAGTGGCGGACGGGTGAGTAATGTCTGGGAAACTGCCT
GATGGAGGGGGATAACTACTGGAAACGGTAGCTAATACCGCATAACGTCGCAAGACCATTGAGGGGGACCTTCGGGCCTCTT
GCCATCGGATGTGCCCAGATGGTATTAGGTTGTTGGTGGGGTAACGGCTCACCTAGGCGACGATCCCTAGCTGGTCTGAGAGG
ATGACCAGGCACACTGGAACTGAGACACGGTCCATACTCCTACGGGAGGGATCAGTGGGGAATATTGCACAATGGGCGCAAG
CCTGATGCAGCCATGCCGCGTGTATGAAGAAGGGCTTCGGGTTGTATAGTACTTTCAGCGAGGAGGAAGGGAGTAAAGTTAA
TACCTTTGCTCATTGACGTTACCCGCAGAATAAGCACCTGCTAACTCCCTGCCAGCTGCCGCGGTAATACGGAGGGTGCAAGC
GTTCTTCGTAATTACTGTGCGTAGAGGGCACGCAGGCGGTTTGTTAAGTCGGATGTGATATCCCCGGGCTCAACCTGGGAACT
GCATCTGATACTGGCAAGCTTGAGTCTCGTAGAGGGCGGTAGAATTCCGTGTGTAGCGGTGAAATGCATATAGATCTGGAGG
AATACCGGTGGCGAAGGCTGCCCCCTGGACGAAGACTGACGCTCATGTGCGAAAGCGTGGATCAGCATACTATGATTATATA
CCCTGGTAGTCCACGCCGTAAACGATGTCCACTTGGAGGTTGTGCCCTTGAGGCGCGGCTTCAGGAGCTCACGCGTTTGTCCA
CCGCCTG

|CP015995.1| Escherichia coli strain S51, complete genome 91% CP015995.1

Figure 10. Complete genome sequence of PCR product of 16S rRNA gene of isolate S30 from well water sample

Figure 12 showed the phylogenetic tree of the isolates which delineates the genetic relatedness of the different isolates from different drinking water sources. The phylogenetic tree showed two different clusters. The first cluster comprises S152, S177, S89, S71 which were identified as *E. coli* strain C-XIB, BAC064, OZK2, S51 respectively while the second cluster comprises S33, S31, S30, S34, S35 which were identified as *E. fergusonii* strain CGS24, *E. coli* strain 268-78-1, S51, OZK2, *E. fergusonii* strain CGS24 respectively.


Escherichia coli-2
Escherichia coli-4
Escherichia coli
Escherichia coli-5
Escherichia coli-3
Escherichia fergusonii
Escherichia coli-7
Escherichia coli-6
Escherichia fergusonii-2
Consensus
Conservation


Escherichia coli-2
Escherichia coli-4
Escherichia coli
Escherichia coli-5
Escherichia coli-3
Escherichia fergusonii
Escherichia coli-7
Escherichia coli-6
Escherichia fergusonii-2
Consensus
Conservation
CCAGCTGCCG CGGTAATACG GAGGGTGCAA GCGTTCTTCG
TAATTACTGT GCGTAGAGGG CACGCAGGCG GTTTGTTAAG
TCGGATGTGA TATCCCCGGG CTCAACCTGG GAACTGCATC
TGATACTGGC AAGCTTGAGT CTCGTAGAGG GCGGTAGAAT
TCCGTGTGTA GCGGTGAAAT GCATATAGAT CTGGAGGAAT
ACCGGTGGCG AAGGCTGCCC CCTGGACGAA GACTGACGCT
CATGTGCGAA AGCGTGGATC AGCATACTAT GATTATATAC
CCTGGTAGTC CACGCCGTAA ACGATGTCCA CTTGGAGGTT
GTGCCCTTGA GGCGCGGCTT CAGGAGCTCA CGCGTTTGTC
CACCGCCTGG GGTGTACGGC CGCTAAGTTA AAACTGTATG
TATTGAGTAG CTGCACCGCA CAAGCGGTGG AGTTGCTAGC

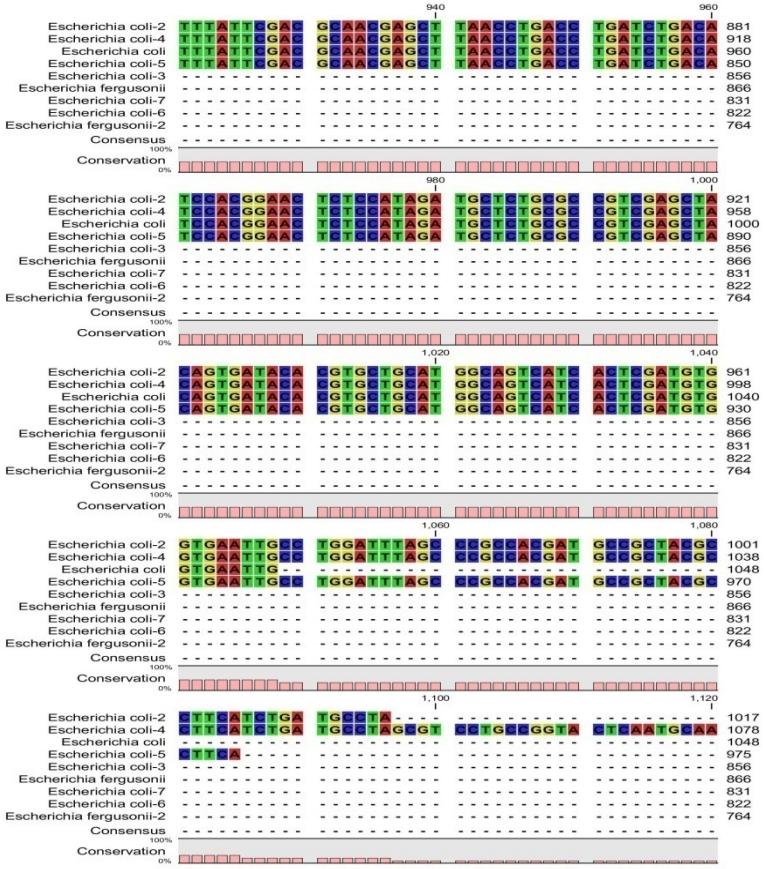


Figure 11. Alignment for the bacteria isolates

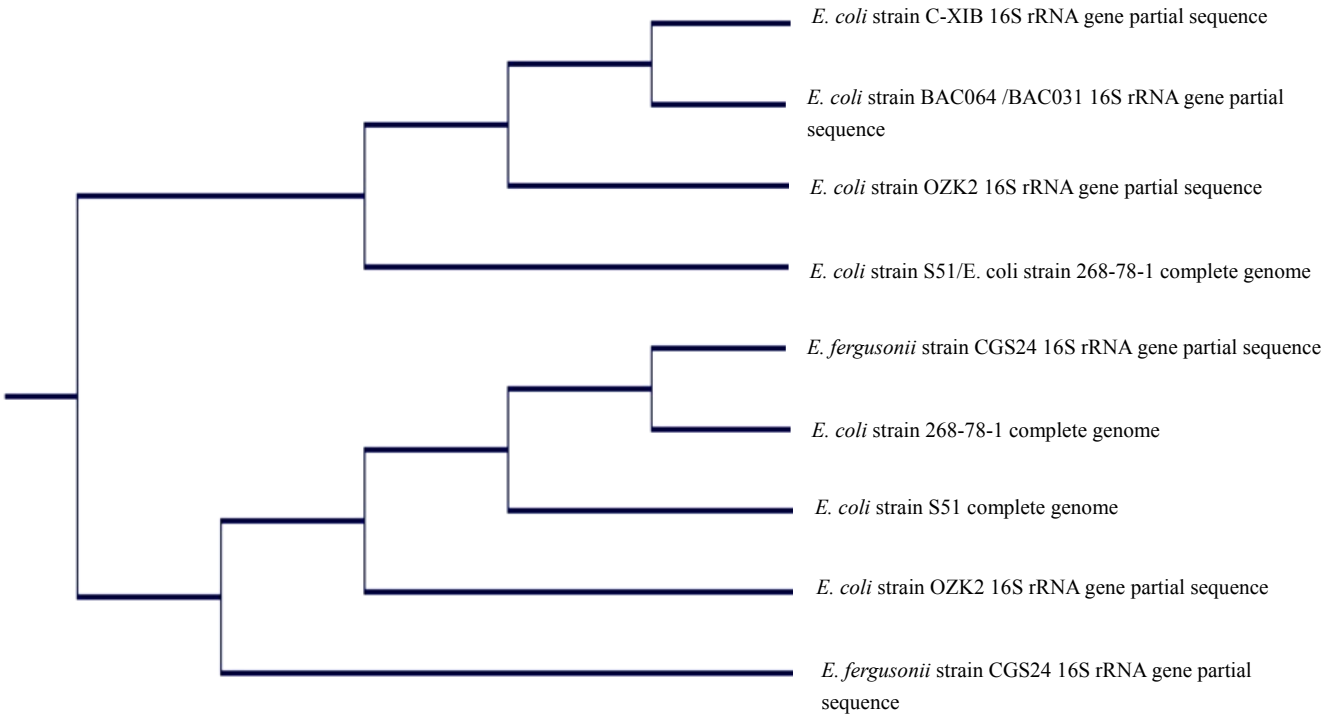


Figure 12. Phylogenetic tree of identified isolates from drinking water sources in Ado-Ekiti and environs

Table 1. Molecular Identification of Bacterial Isolates from Drinking Water Samples in Ado-Ekiti and Environs using 16S rRNA

Bacterial isolates	Source	Strain to which isolate was similar to in the gene bank	Similarity (%)	NCBI gene bank accession number
S71	Well	*E. coli* strain S51 and	91%	CP015995.1
		E. coli strain 268-78-1	91%	CP014092.1
S177	Stream	*E. coli* strain BACO64 and	91%	KU161315.1
		E. coli strain BAC031	91%	KU161312.1
S31	Borehole	*E. coli* strain 268-78-1	91%	CP014092.1
S89	Well	*E. coli* strain OZK2	91%	KT440880.1
S152	Well	*E. coli* strain C-XIB	89%	KJ806504.1
S34	Pipe-borne	*E. coli* strain OZK2	91%	KT440880.1
S33	Borehole	*E. fergusonii* strain CGS24	91%	KX034239.1
S35	Well	*E. fergusonii* strain CGS24	91%	KX034239.1
S30	Well	*E. coli* strain S51	91%	CP015995.1

4. Discussion

The traditional identification of bacteria on the basis of phenotypic characteristics is not as reliable as recent identification which is based on genotypic methods. The ribosomal RNA sequence based analysis is an implicit and unique method to understand microbial diversity within and across a group and also to identify new strains (Magray *et al*., 2011). Bacterial species have at least one copy of the 16S rRNA gene containing highly conserved regions together with hyper variable regions, which is used for identification of new strains. The comparison of the bacterial 16S rRNA gene sequence with a known bacterial sequence in the database has emerged as a preferred genetic technique. Also, the sequence of the 16S rRNA gene has been widely used to estimate phylogenetic relationships among bacteria (Sujatha *et al.,* 2012).

The results of identification in this study showed an accurate and definitive identification of the isolates. This revealed that all the isolates were of *Escherichia* genus. While seven from nine isolates were identified as *E. coli,* two were identified as *E. fergusonii.* The study unveiled the shortcoming of traditional/conventional identification which is based on phenotypic characteristics. Furthermore, the inability to differentiate *E. fergusonii* from *E. coli* in the previous study also corroborates findings by Wragg *et al.* (2009). Their study showed that *E. fergusonii* isolates possess both some phenotypic and genotypic features linked to pathogenic *E. coli* and also support the evidence that strains of *E. fergusonii* may act as opportunistic pathogens. In another study by Walk *et al*. (2009), they also unveiled cryptic lineages of the genus *Escherichia*, which shared similar phenotypic traits, by using molecular tool.

5. Conclusions

To the best of our knowledge, this study reports the incidence of *E. fergusonii* in drinking water sources in Nigeria for the first time. Therefore, the use of 16S rRNA gene sequencing in this study has contributed immensely in giving a decisive differentiation and identification of similar bacterial isolates belonging to the same genus.

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