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Goalpara and bacterial water contamination: A thorough study

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***Abstract*---**The current world is fighting with several issues like Covid-19, Poverty, Malnutrition and many more. But there are some other issues which are still ignored by considering as less important. Among these least concern issues death due to drinking of contaminated water or ingestion of food come in contact with polluted water holds a major percentage. The study was conducted to determine the quality of water contamination trend in Goalpara district of Assam. The presence of fecal and total coliform were tested by Most Probable Number as well as Membrane filtration tests (MF) to rule out any chance of missing out any positive sample. During the study we identified the causative agent which contaminates the water in the targeted district of Assam, India. Water samples were collected from drinking water supplies and from routine household chores. During the study, evidence of microorganisms such as Escherichia coli, Streptococci, Staphylococci and Klebsiella were found. Escherichia coli were isolated from most numbers of samples, whereas Shigella spp. has the lowest contamination rate. The water with highest contamination rate was from Rangjuli development block and Lakhipur development block gave the lowest water contamination rate. The most prevalent bacteria were Escherichia coli. During the summer and Monsoon season the rate of contamination for both the year 2019 and 2020 were higher i.e. 80% and 87% respectively. Again, the rate of contamination is recorded lower in the winter season of both the years i.e. 20% and 33.3% respectively. Poor sanitation, lack of understanding about basic hygiene and cleanliness, poor economic conditions, hygiene carelessness, and other activities contribute to water contamination in

the district. Bacteria in water raise the risk of waterborne illnesses and other health issues.

***Keywords*---**bacterial contamination, coli form bacteria, drinking water, membrane filtration, water borne diseases.

Introduction

Goalpara is a wonderfully designed area on the southern edge of the Brahmaputra River. The district has an area of 1842 square kilometres. It is surrounded on the south by Meghalaya's Western and Eastern Garo hills districts, and on the east by Kamrup district of Assam. Similarly, the West half shared a boundary with district Dhubri, while the powerful River Brahmaputra runs across the northern. The district is situated here between latitudes of 25^0 53′ and 26^0 15″ north and the longitudes of 90^0 07′ and 91^0 05″ east. [4]. It is a town in Assam's western region. The evergreen forests of the lower mountains provide such a hilly scene. The district of Goalpara features more attractive lower plains and hills. Pancharatna, Sri Surjya, Tukreswari, Nalanga, and Paglartek are the major hills with heights ranging from 100 to 500 metres. Hulukanda Hill is located near the Brahmaputra River in Goalpara. The hill is also home to numerous rare flora, plants, and streams, as well as a variety of wild birds.[1] The Brahmaputra River travels east to west, including tributaries including the Dudhnoi , Krishnai and Jinjiram River. The Dudhnoi and Krishnai rivers originate in the Meghalaya Hills and drain into the Brahmaputra as that of the Mornoi River west of Matia. The Jinjiram originates in Urpad Beel and goes in tandem with the Brahmaputra before reaching the South Salmara region. There are just few additional minor streams in the district, as well as various Beels (natural reserve forests, lakes) such Urpad Beel, Hashila Beel, Kumri Beel, and Dhamar Risan Beel, and several more manmade ponds. The Urpad Lake has become a migrating birds hotspot from October till March. Kumri Lake is an important fish-growing area out of which fish are supplied to Assam's different markets. [2]. Krishnai, Kuchdhowa, Balijana, Kharmuza, Jaleswar, Lakhipur, Rangjuli, and Matia are the eight development blocks in total.

Goalpara is one of Assam's poorest districts in terms of the Human Development Index (HDI). With an index value of 0.308, the district ranks 18th in terms of HDI, well below the state average of 0.407. In terms of wealth, education, and health, the district ranks 14th, 18th, and 16th in the district, respectively. According to the Human Poverty Index from 1999, 26.30 percent of the district's population lives in poverty. Goalpara is a flood prone district. [14]

Figure 1: District Map for Goalpara with borders

The infectious diseases caused by pathogenic bacteria, viruses and parasites are the most common and widespread health risks associated with drinking water in rural habitation. [7, 8] The quality of the drinking water can be checked by its microbial examination. [6]

The aim of this study was to observe the quality of drinking water and the pattern of bacterial contaminants prevalent in the area. Again, this study tried to find out the area wise rate of bacterial contamination of drinking water. Further, the most prevalent bacteria was confirmed with the help of 16srRNA molecular identification followed by Gel Electrophoresis method.

Materials and Methods

Study Area

The study was conducted in Goalpara district of Assam. Under Goalpara district eight development blocks namely, Balijana, Krishnai, Kharmuza, Rangjuli, Jaleswar, Lakhipur, Matia, Kuchdhowa comes. The total population of the district is a about 10,08,183 according to the 2011 census which ranked in 10th position in population at state level and constitutes 3.23% of state population. [3] Rain, rivers, and streams are the principal water sources in this region, which also shares a border with Meghalaya, a mountainous state. [5]

Sample Collection and Processing

120 samples were collected in a wide mouthed sterile sample collection bottle with a 1 L capability following WHO standard protocols from various sources of drinking water with in Goalpara.[12] The water was collected in an aseptic

manner, and sample pickers were required to wear proper personal protective equipment while doing so.[13] While transferring the sample to a laboratory for the further analysis, the sample bottles were kept in an airtight huge ice box at a temperature of 2-8^0 C.[11]

Both Membrane filtration and Most Probable Number test is done to identify the positive samples. [3] Both the tests are performed to retain any chances to miss out any sample with bacterial contaminants. On the basis of morphological, cultural, and biochemical characteristics, suspected clusters of coli form groups are further identified. [9] Microbes were isolated by spread plate method. [10] Isolated bacteria were identified with the help of various biochemical tests.

In the membrane filtration method the membrane filters after filtration of the water sample were inoculated in Modified Tergitol 7 agar medium and incubated at 37 degree Celsius and 44 degree Celsius (in water bath) to grow and differentiate between the coli form, non- coli form and thermotolarent coli form bacteria. Further, selective media such as Chromogenic Coliform Agar were used for isolating coli form bacteria. When oxidase negative colonies develop acid and gas in lactose peptone water broth incubated at 44 ^{0} C and indole in tryptone water broth incubated at 44^0 C, they are recognized as E. coli. The result may be used to compute the validated E.coli and coli form counts per 100 ml of water sample. [15] Sample collection duration was two years i.e. 2019 and 2020. All the four seasons were cover during the study.

Results

Membrane filtration was determined to be positive in 62 of the 120 water test samples. From diverse sample sources, Escherichia coli, Klebsiella, Staphylococcus, Streptococcus, Enterococcus, and Shigella are isolated.

Table 1:- Block wise rate of Positivity

Sl no	Blocks	No of sample tested	No of sample found Positive	% of positivity block wise
1	Balijana	15	11	73.3
2	Krishnai	15	8	53.3
3	Kharmuza	15	7	46.6
4	Rangjuli	15	13	86.6
5	jaleswar	15	6	40
6	Lakhipur	15	4	26.6
7	Matia	15	7	46.6
8	Kuchdhowa	15	6	40

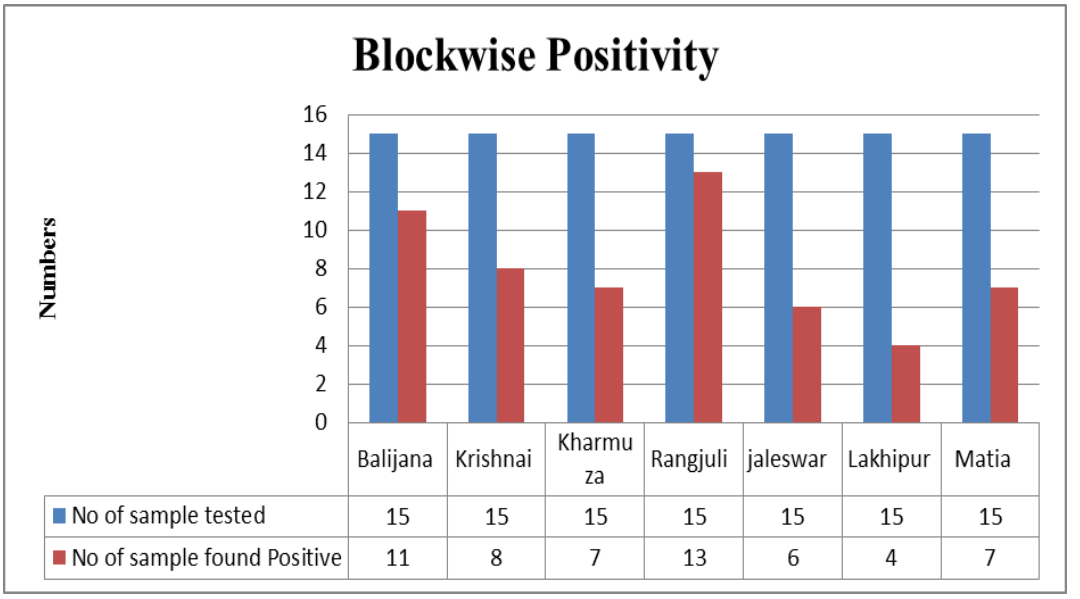

Figure 2:- Block wise Number of Positive sample

Escherichia coli were reported with highest percentage i.e. 32.25% and Shigella were reported with 1.61% which is lowest in our study.

Table 2:- Number of Bacteria isolated in Blocks

Name of bacteria	Blocks								Total	% of bactria on positive samples
	Rangjuli	Balijana	Krishnai	Kharmuza	Jaleswar	Lakhipur	Matia	Kuchdhowa		
E.coli	2	5	2	3	2	0	2	4	20	32.25
Klebsiella Spp.	2	1	1	0	0	0	0	0	4	6.45
Staphylococcus Spp.	2	0	0	0	0	1	2	0	5	8.06
Streptococcus Spp.	5	3	2	2	2	2	1	2	19	30.64
Enterococcus Spp.	2	1	3	2	2	1	2	0	13	20.96
Shigella Spp.	0	1	0	0	0	0	0	0	1	1.61

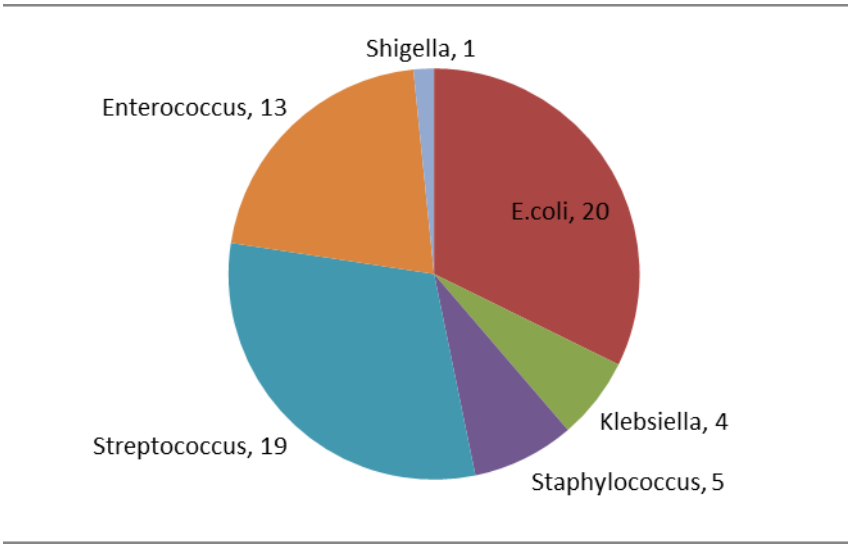

Figure 3:- Numbers of Bacteria isolated

Kuchdhowa development block did not provide any evidence of Enterococcus isolates. Except Lakhipur, the bacteria, E.coli were isolated from all of the blocks. The highest number (5) of this bacterium was found in the Balijana block. The development blocks Rangjuli (2), Balijana (1), and Krishnai (1) reported presence of klebsiella, whereas staphylococci were isolated from Rangjuli (2), Lakhipur (1), and Matia (2). Streptococcus were isolated from all of the blocks, however Shigella was only found in Balijana (1) block.

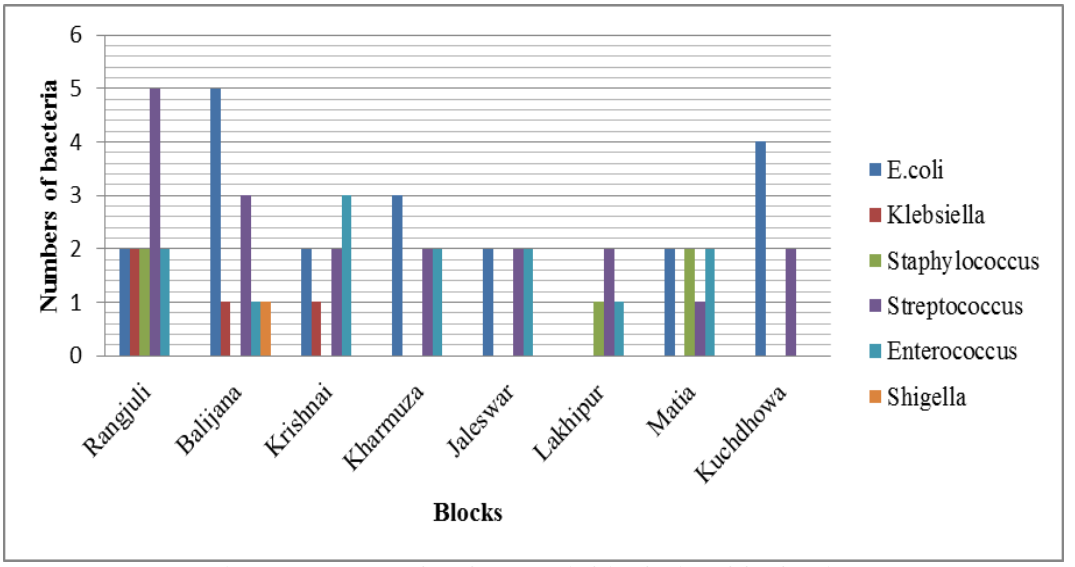

Figure 4: - Trends of Bacterial isolation block wise

Table 3:- Number of Prevalent bacteria

Blocks	Prevalent bacteria	Number of prevalent bacteria	% of prevalent bacteria on total positive sample	Block wise % of positivity of the prevalent bacteria
Rangjuli	Streptococcus	5	8.06	33.33
Balijana	Escherichia coli	5	8.06	33.33
Krishnai	Enterococcus	3	4.83	20
Kharmuza	Escherichia coli	3	4.83	20
Jaleswar	Escherichia coli, Streptococcus, Enterococcus	2	3.22	13.33
Lakhipur	Streptococcus	2	3.22	13.33
Matia	Escherichia coli, Staphylococcus, Enterococcus	2	3.22	13.33
Kuchdhowa	Escherichia coli	4	6.45	26.66

In the study when the prevalence of bacteria was observed it was found that seven out of eight development blocks were with water sources contaminated with Escherichia coli. Streptococcus was prevalent in Rangjuli (33.33%), Balijana (33.33%) and Lakhipur (13.33%). Again, Escherichia coli were found prevalent in Balijana (33.33%), Kuchdhowa (26%) and Kharmuza (20%). Enterococcus was recorded in Krishnai(20%) as the prevalent bacteria isolated. Jaleswar showed prevalence of Escherichia coli, Streptococci and Enterococcus with a percentage of 13.33% whereas Matia block gives the same figure denoting prevalent bacteria as Escherichia coli, Staphylococcus and Enterococcus.

Table 4:- Season wise Positivity

Year	Seasonal rate of positivity								Total and percentage on positive sample
	March-April/Spring		April-August/ Summer and Monsoon		September- October/Autumn		October- March/Winter		
	No of sample collected	No of sample found positive	No of sample collected	No of sample found positive	No of sample collected	No of sample found positive	No of sample collected	No of sample found positive	
2019	15	9(60%)	15	12(80%)	15	4(27%)	15	3(20%)	28(45.16%)
2020	15	8(53.3%)	15	13(87%)	15	8(53.3%)	15	5(33.3%)	34(54.83%)

15 numbers of samples were collected in each season of both the years where the rate of contamination is little higher in the year 2020 (54.83%) in comparison to 2019(45.16%).

In the year 2019 the rate of water contamination was 60%, 80%, 27% and 20% for spring, summer and Monsoon, autumn and Winter respectively. Again for the year 2020 the contamination rate for all the four seasons were 53.3 %(spring), 87 %(summer and monsoon), 53.3 %(autumn) and 33.3 %(winter) respectively.

Figure 5: Coli form bacteria in Modified Tergitol 7 Agar Media with Membrane Filter

Figure 6: Lone colonies of E.coli in CCA media

Figure 7: Positive MPN tubes

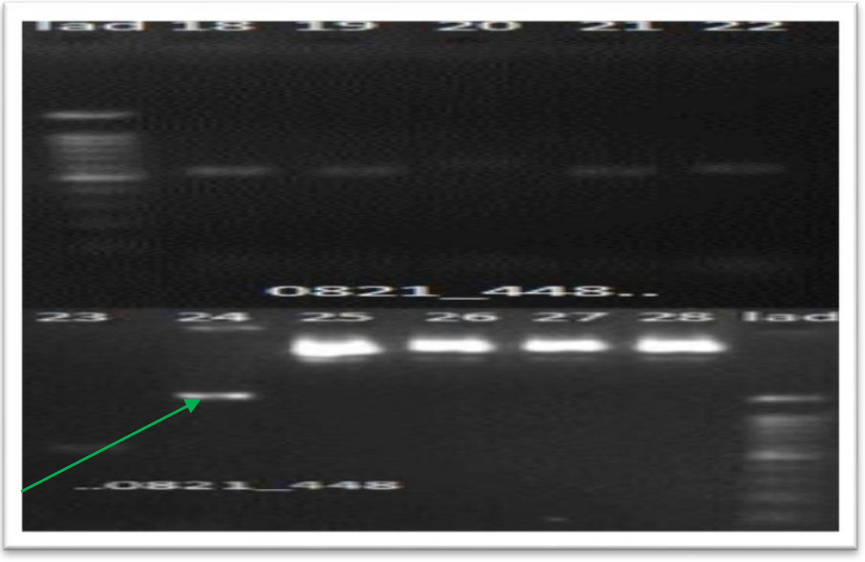

Figure 8:- Electrophoresis image of targeted organism

As Escherichia coli was isolated from highest number of water samples the bacteria was tested by Polymerase Chain Reaction followed by sequencing and Electrophoresis. And, these series of tests confirms that the isolated bacterium was Escherichia coli.

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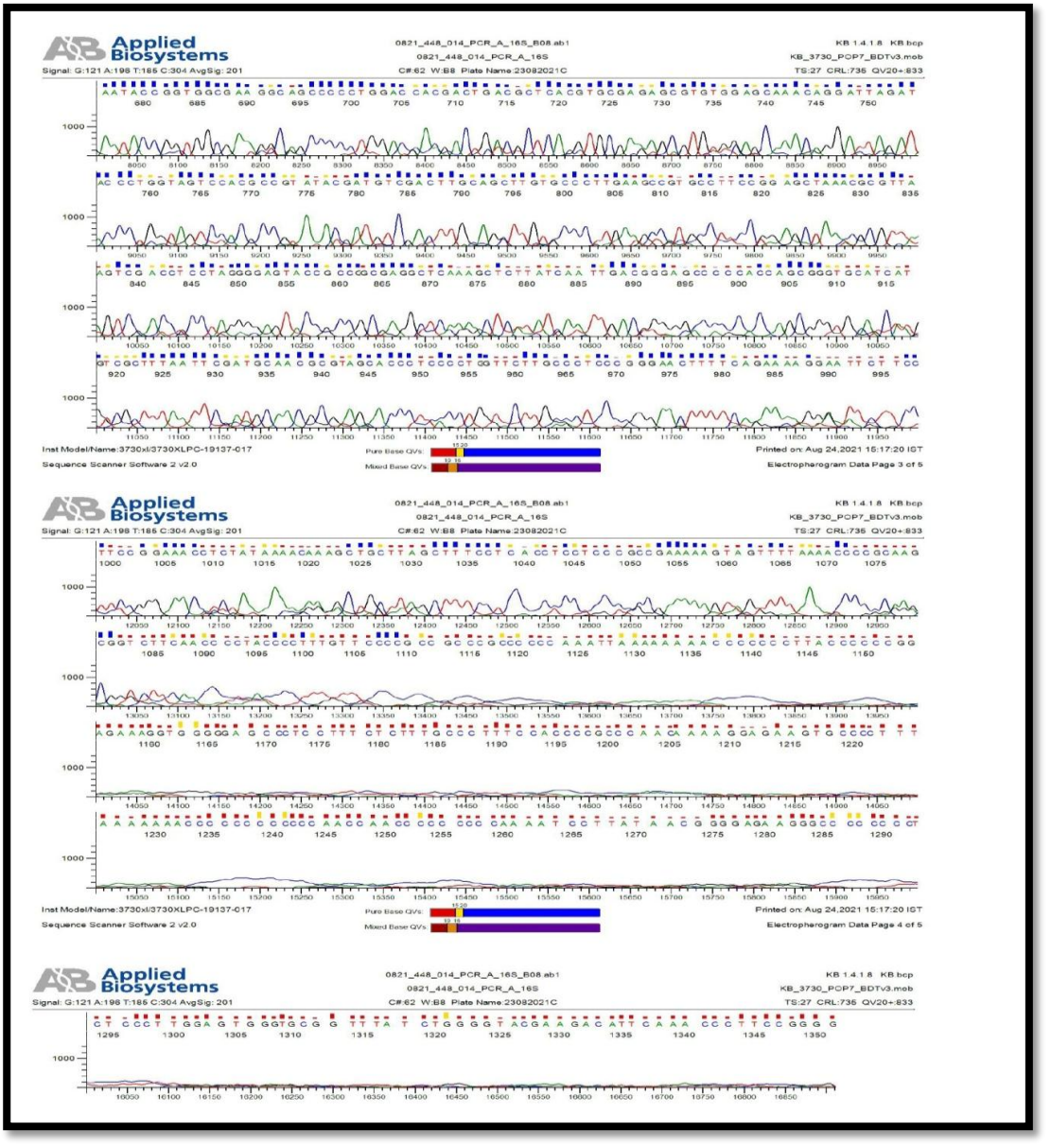
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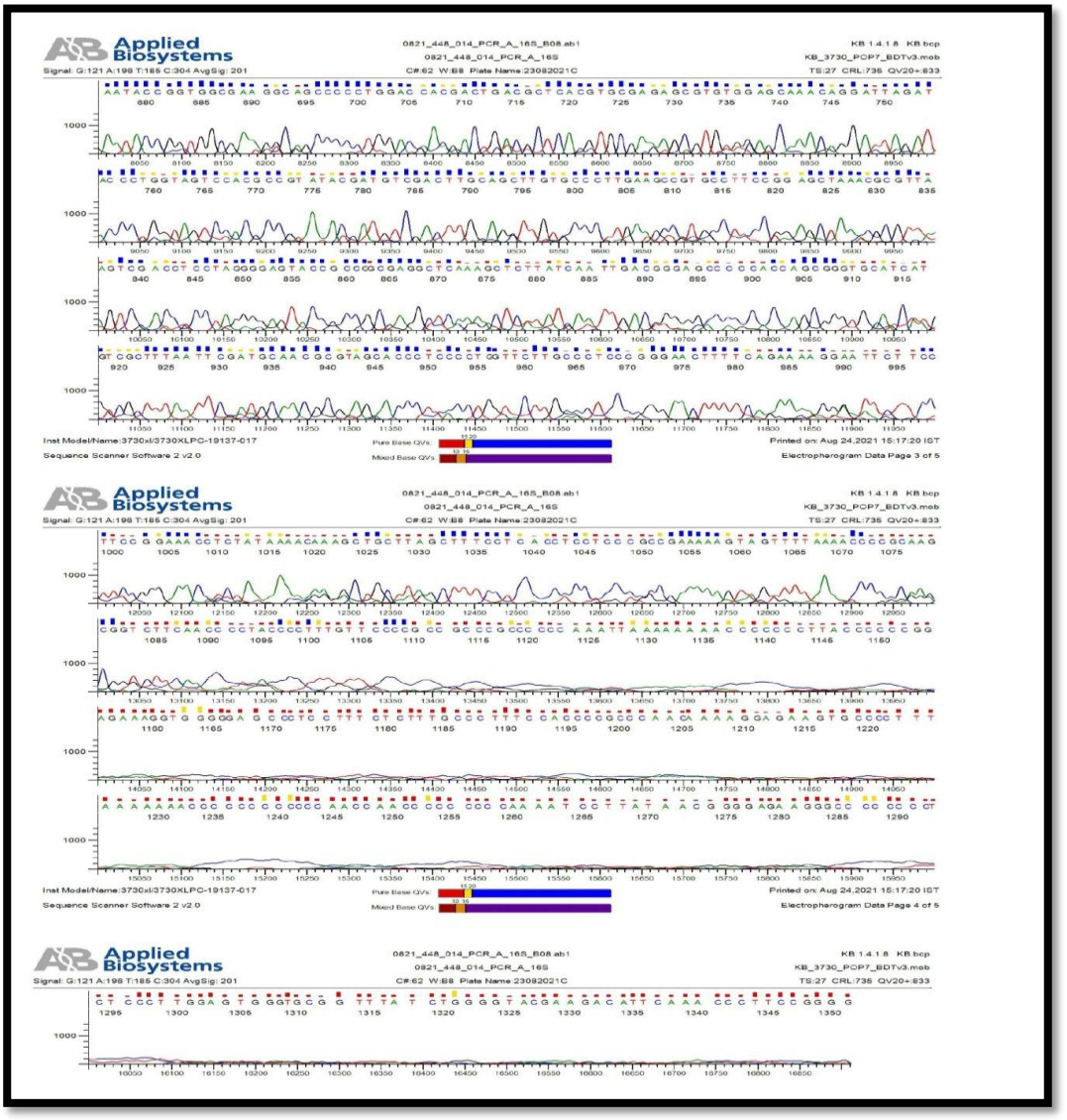

Figure 9: Whole DNA Sequence of the prime bacteria isolated

Discussion

Traces of water contamination were observed in every part of the Goalpara district. Both total coliform and fecal coliform were isolated from various development blocks. These findings emphasise the importance of paying special attention to household pollution, hygienic conditions, and increasing awareness about water contamination. As indicated in Table 1, the highest rate of pollution was found in the Rangjuli block, followed by Balijana, Kharmuza, Kuchdhowa, Jaleswar, Krishnai, and Lakhipur. Most of the water samples obtained in the research included E.coli as the predominant bacterium. Klebsiella, Enterococcus, Streptococcus, Staphylococcus, and Shigella were among the other species recovered, as shown in Table 2. E.coli was determined to be the most common

bacterium in a research done by Yassir Mohammed Eltahir and Amira Ahmed in South Darfur, Sudan. Similarly, James Okot Okumu and Jaocob Otim discovered E.coli to be the most often isolated bacterium in a research done in various Ugandan locations. During the two year study highest rate of contamination was recorded in summer &monsoon season of both the year 2019 (80%) and 2020 (87%). Similar findings were recorded in a study conducted in Khairpur City of Pakistan by Shar et al. where the rate of contamination were higher in summer season.

Conclusion

Water contamination is a serious issue since it is connected to a number of water-borne ailments. The quality of drinking water in the Goalpara region, according to the statistics, is filthy and substandard. We must focus on increasing sanitation practises by raising awareness about the necessity of hand washing, enclosing a well or reservoir, drinking treated or boiling water, and using filters, among other things, because the bulk of these specimens were collected in rural areas. In some areas, the same water source is used for drinking, as well as for domestic duties such as washing clothing and dishes and bathing cattle. As a consequence, by applying appropriate sanitary practices, we may improve the quality of water and, as a result, the level of living for Goalpara people.

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