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Screening of foodborne pathogens from selected food samples

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Abstract
In the present study, a total number of 191 bacterial isolates were isolated from selected food samples. Samples were categorized as fruit, vegetable, poultry, and dairy samples. Maximum occurrence of pathogens was recorded in dairy samples *i.e.*, 52 isolates followed by vegetable samples (49 isolates), poultry samples (47 isolates) while the minimum occurrence of pathogens was recorded in fruit samples (43 isolates). The isolates were identified on the basis of their cultural characteristics, morphological analysis, biochemical test, and molecular identification. Molecular identification was done by 16s rRNA sequencing. Among 191 isolates, 57 were identified as *Escherichia coli*, 51 were *Staphylococcus aureus*, 13 isolates were *Shigella dysenteriae* and 14 isolates were identified as *Salmonella typhi*.

Keywords: Foodborne pathogen, *Staphylococcus aureus, Escherichia coli, Shigella dysenteriae, Salmonella typhi*

Introduction
Food-borne diseases occur as a result of the consumption of contaminated food or water (Tassew *et al.*, 2011; Assefa and Bihon, 2018; Faris, 2015 and Haile *et al.*, 2017) [38, 10, 21, 22]. Contamination is occurring due to pathogens or their toxins. The pathogens which caused foodborne diseases are referred foodborne pathogens. These pathogens include bacteria, viruses, fungi, and parasites (Zhao *et al.*, 2014) [47]. In both developed and developing countries, foodborne diseases are major health problems. According to WHO, in developed countries, every year ~30% of the population suffers from foodborne diseases, and in developing countries, up to 2 million deaths per year are estimated because of foodborne diseases (Abunna *et al.*, 2016) [2]. Currently, the foodborne disease caused by bacterial pathogens is one of the biggest issues in developing countries as it causes economic loss in addition to public health and food safety. The pathogenicity of bacteria is dependent on their capacity to produce toxins. Bacteria are the causative agent of two-thirds of foodborne disease outbreaks (Argaw and Addis, 2015) [9]. The common foodborne bacterial pathogens are *Staphylococcus aureus, Salmonella species, Shigella spp., Listeria monocytogenes, Campylobacter jejuni, Vibrio cholerae, Clostridium perfringens, Bacillus cereus, Yersinia enterocolitica, Clostridium botulinum, Micrococcus luteus, Proteus vulgaris, Enterobacter aerogens, Klebsiella pneumoniae, Bacillus megaterium* and *Escherichia coli.* These bacteria may enter into the food chain from the production of food up to the final consumption of food products. Most of *Escherichia coli* are normal inhabitants of the human gastrointestinal tract (Disassa *et al.*, 2017; Abreham *et al.*, 2019 and Taye *et al.*, 2013) [18, 1, 39], while rest are pathogenic to humans (Bekele *et al.*, 2014) [13]. Pathogenic *Escherichia coli* are distinguished from normal inhabitants by their possession of virulence factors. Based on virulence factors pathogenic *Escherichia coli* are categorized into five virulence groups, namely, enter aggregative *Escherichia coli*, enter hemorrhagic *Escherichia coli*, enter invasive *Escherichia coli*, enteropathogenic *Escherichia coli*, and enterotoxigenic *Escherichia coli* (Assefa and Bihon, 2018) [10]. *Escherichia coli* are also considered a reliable indicator of contamination by manure, soil, and contaminated water (Disassa *et al.*, 2017) [18].
Staphylococcus aureus is a well-known opportunistic foodborne pathogen (Rodrıguez-Lazaro *et al.*, 2017 and Wang *et al.*, 2017) [45]. It has a broad host range including human beings and food-producing animals like pigs, cows, goats, chickens, and ducks (Wang *et al.*, 2017) [45]. Staphylococcal food poisoning is caused by the consumption of contaminated food with staphylococcal entero-toxins (EI-Jakee *et al.*, 2013) [19]. *Staphylococcus aureus* causes food contamination directly from infected food-producing animals. On the other hand, contamination may also be caused by poor hygiene during the production, processing, retailing, or storage of food products (Massawe *et al.*, 2019) [30].

Foodborne disease caused by *Shigella dysenteriae* is recognized as a serious health problem throughout the world. It is highly infectious because a low infectious dose of *Shigella dysenteriae i.e.*, 10-100 organisms is enough to cause disease. (Patil and Lava, 2012) [32]. *Shigella dysenteriae* is an enteric pathogen and frequently associated with food and waterborne diseases which leads to acute invasive infections. In developing countries, it is found most frequently in environments of compromised sanitation, improper waste management, unsafe drinking water, and poor hygienic condition. Lack of proper access to food sources and poor health care contribute to a high risk of morbidity and mortality. It is transmitted through the fecal-oral route and by direct contact with an infected individual.

Salmonella typhi is an important foodborne pathogen in most countries of the world especially in developing countries (Soultose *et al.*, 2003; Carraminana *et al.*, 2004) [36, 15]. It causes mortality and morbidity due to water and foodborne diseases in almost all countries causing human gastroenteritis and typhoid fever (Malorny *et al.*, 2008) [29]. It accounts for ~93.8 million foodborne illnesses and 155,000 deaths per year worldwide (Heredia and Garcia, 2018) [24]. Food sources of *Salmonella typhi* include mainly milk, eggs, meat (poultry, beef), vegetables, and fresh fruits (Almeida *et al.*, 2013) [6].

Taking into consideration the importance of isolation and identification of foodborne pathogens, the present study aimed at isolation and identification of four foodborne pathogens namely, *Escherichia coli, Staphylococcus aureus, Salmonella typhi,* and *Shigella dysenteriae* from selected food samples. The present work will act as foundation work to detect the prevalence and contamination levels in selected food samples. This will help in protection of food products from decaying and deterioration and help to come up with strategies to minimize the risk of spreading food borne disease.

Material and Method

Place of the work

All the experiments were conducted in Dairy Food Quality and Safety Lab, Department of Dairy Microbiology, Warner College of Dairy Technology, Sam Higginbottom University of Agriculture, Technology and Sciences, Prayagraj, Uttar Pradesh (India) during the year 2021.

Isolation of pathogens

For isolation of foodborne bacterial pathogens selected food samples (dairy products, poultry products, fruit, and vegetable) were collected from 10 localities of Prayagraj, Utter Pradesh. Isolation was done in Nutrient agar media by following the conventional method (Aneja, 2003) [8]. Primary confirmation was done by streaking pure culture on selective agar media. Mannitol Salt Agar (M.S.A.) media for *Staphylococcus aureus*, Eosin Methylene Blue (E.M.B.) agar media for *Escherichia coli*, Xylose Lysine Deoxycholate (X.L.D.) agar for *Salmonella typhi,* and Salmonella-Shigella Agar (S.S.A.) media for *Shigella dysenteriae* was used.

Identification of isolated pathogens

Cultural and morphological identification

On the respective agar plates, cultural characteristics of isolates (colonial appearance, size, elevation, form, edge, consistency, colour, odour, opacity, and pigmentation) were recorded. For morphological identification Gram's staining was done by following the standard procedure of staining (Aneja, 2003) [8].

Biochemical identification

Biochemical identification of isolates was done by performing specific biochemical tests. The performed tests were the Catalase test, Oxidase test, Indole test, Methyl Red test, Voges Proskauer test, Citrate test, Nitrate reduction test, Urease test, Carbohydrate fermentation tests**.** All the tests were done by following standard procedures (Cappuccino and Sherman, 2005) [11].

Molecular identification

Molecular identification of isolates was done in Scangene Labs Pvt. Ltd., Delhi by the Sanger sequencing method.

Results and Discussion

Isolation of food borne bacterial pathogens

The conventional cultural method is considered the "gold standard" for the isolation and identification of foodborne pathogens. This method consists of a series of steps which includes nonselective enrichment followed by selective enrichment, selective or differential plating, and finally identification by morphological, biochemical, and serological confirmation. In the present study, the conventional cultural method was used for the isolation of pathogens. A total of 191 foodborne bacterial isolates were isolated from 200 samples, of which 57 were *Escherichia coli,* 51 were *Staphylococcus aureus,* 38 were *Shigella dysenteriae* and 45 were *Salmonella typhi*. Previously Akter *et al.*, (2013) [3], Alwan and Talak (2015) [7], Chen *et al.*, (2018) [16], and Dai *et al.*, (2019) [17] also used the same method in their study.

Isolates were designated according to the source of isolation. Isolates from the fruit samples were designated as F_1 to F_{43}. Among 43 isolates, 10 were *Escherichia coli,* 15 were *Staphylococcus aureus,* 8 were *Shigella dysenteriae* and 10 were *Salmonella typhi.* A total of 49 isolates, isolated from the vegetable samples, were designated as V_1 to V_{49}, among which 12 were *Escherichia coli,* 14 were *Staphylococcus aureus,* 11 were *Shigella dysenteriae* and 12 were *Salmonella typhi.* 52 isolates from dairy samples were designated as D_1 to D_{52}, in which 15 were *Escherichia coli,* 10 were *Staphylococcus aureus,* 13 were *Shigella dysenteriae* and 14 were *Salmonella typhi.* 47 isolates isolated from poultry samples were designated as P_1 to $P_{47,}$ in which 20 were *Escherichia* coli, 12 were *Staphylococcus aureus,* 6 were *Shigella dysenteriae* and 9 were *Salmonella typhi.* (Table 1 and Fig. 1). The percentage of incidence of foodborne pathogens in the collected sample was recorded. The results of the present study were more or less in agreement with the findings of previous researches. In the present study, *Escherichia coli* and *Staphylococcus aureus* were found in 29.84% and 26.70% of the samples, respectively. In both cases, the findings are higher than the research by Alharbi *et al.*, (2019) [4] and Hemalata and Virupakshaiah (2016) [23] but lower than Bantawa *et al.*, (2018) [12]. In 19.90% of samples, *Shigella dysenteriae* was recorded. This finding was higher than the research by Bantawa *et al.*, (2018) [12]. Similarly, *Salmonella typhi* was recorded in 23.56% samples which is higher than the research by Alharbi *et al.*, (2019) [4] and Hemalata and Virupakshaiah (2016) [23] but lower than Islam *et al.*, (2014) [25]. The differences among the percentage of incidence might be due to the sample variation, geographic variation, species differentiation, and technical limitations of the laboratory where the study was performed.

Table 1: Foodborne pathogenic bacterial pathogens and their source

Total No. of Samples (n=200)	No. of isolated pathogens			
	Escherichia coli	*Staphylococcus aureus*	*Shigella dysenteriae*	*Salmonella typhi*
Fruits (n=50) (Apple, pomegranate, Grapes, Papaya, Guava)	10	15	08	10
Vegetables (n=50) (Beans, ladyfinger, spinach, chilly, coriander, tomato, capsicum, peas, cabbage, cauliflower, carrot, potato, onion, ridge guard, brinjal, bitter guard, cluster beans)	12	14	11	12
Dairy products (n=50) (Raw milk, paneer, cheese, butter, curd)	15	10	13	14
Poultry products (n=50) (Chicken meat, goat meat, fish)	20	12	06	09
Total	57	51	38	45

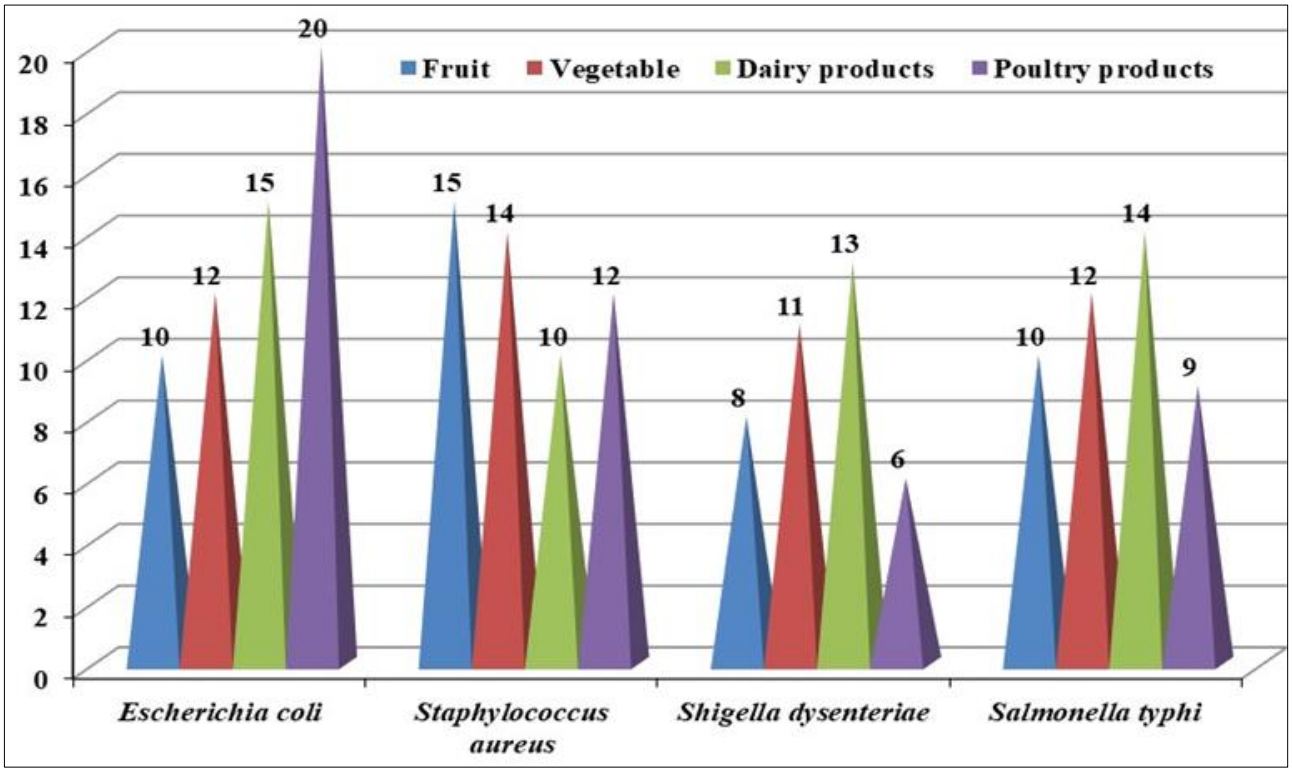

Fig 1: Incidence of pathogens in selected food samples

Morphological identification of isolated bacterial pathogens

All the isolates were identified by morphological characterization on a nutrient agar media plate (Fig. 2). Morphological characteristics of the isolates are shown in table 2. In the present study, the recorded morphological characteristics of *Escherichia coli* were similar to the findings of Thanigaivel and Anandhan (2015) [43], Islam *et al.*, (2014) [25], and Hemalata and Virupakshaiah (2016) [23]. Similarly, morphological characterization and microscopic observation of *Staphylococcus aureus* were similar to the observations of Thanigaivel and Anandhan (2015) [43], Hemalata and Virupakshaiah (2016) [23], Rodríguez-Lázaro *et al.*, (2017) [34], and Pondit (2018) [33]. The recorded colony characterization and microscopic observations of *Shigella dysenteriae* were similar to the previous study conducted by Sheikh *et al.*, (2019) [37] and Saima *et al.*, (2018) [35]. The observations by Hemalata and Virupakshaiah (2016) [23] and Islam *et al.*, (2014) [25] agree with the morphological characterization of *Salmonella typhi* in the present study.

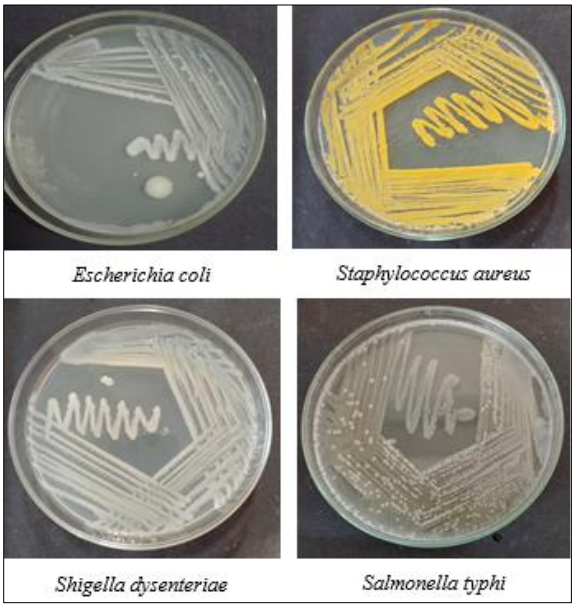

Fig 2: Food borne pathogen isolates on nutrient agar media plate

Table 2: Morphological identification of foodborne pathogenic bacteria isolates

Morphological Characters	Foodborne pathogenic bacteria isolates			
	Escherichia coli	***Staphylococcus aureus***	***Shigella dysenteriae***	***Salmonella typhi***
Colony margins	Entire	Entire	Entire	Entire
Colony form	Circular	Circular	Circular	Circular
Elevation	Flat	Convex	Convex	Raised
Optical density	Opaque	Opaque	Transparent	Translucent
Colony colour	White	Yellow	Colorless	Grayish white
Surface	Glistening	smooth, shiny	Smooth	Smooth and Moist
Microscopic observation				
Gram reaction	Gram –ve	Gram +ve	Gram –ve	Gram –ve
Cell Shape	Rods	Cocci	Rods	Short rods
Arrangement	Scattered Arrangement	bunches	Scattered Arrangement	Scattered Arrangement

-ve = negative and +ve = positive

Biochemical Identification of isolated bacterial pathogens

After morphological identification of isolates, biochemical identification was done by using biochemical tests and carbohydrate fermentation tests (arabinose, fructose, glucose, lactose, maltose, mannitol, raffinose, sucrose, xylose, and sorbitol). The biochemical characterization of isolates is shown in table 3.

The current findings are in agreement with the report of Hemalata and Virupakshaiah (2016) [23] who have found *Escherichia coli*, *Staphylococcus aureus*, and *Salmonella typhi* positive for catalase and nitrate reduction test. Similar reports were recorded in the study of Thanigaivel and Anandhan (2015) [43] for *Escherichia coli* and *Staphylococcus aureus.* The biochemical characteristics of *Shigella dysenteriae* recorded in the present study are similar in research by Sheikh *et al.,* (2019) [37] Saima *et al.,* (2018) [35], and Kinge and Mbewe (2010) [27]. The research by Sedeik *et al.,* (2019) Nair *et al*., (2015) [31], Islam *et al.,* (2014) [25], and Kebede *et al.,* (2016) [26] supports the findings of biochemical characteristics of *Salmonella typhi.* The biochemical characteristics of *Escherichia coli* are also supported by Zinnah *et al.,* (2007) [46], Islam *et al.,* (2014) [25], and Tilahun and Engdawork (2019) [44]. Similarly, the biochemical characterization of *Staphylococcus aureus* is also in agreement with the report of Thaker *et al.,* (2013) [42] Rodríguez-Lázaro *et al.,* (2017) [34], and Pondit (2018) [33].

Table 3: Biochemical characterization of isolates

Isolates	***Escherichia coli***	***Staphylococcus aureus***	***Shigella dysenteriae***	***Salmonella typhi***
Biochemical test				
Oxidase	-	-	+	-
Catalase	+	+	+	+
Methyl red	+	+	+	+
Voges-Proskauer	-	+	-	-
Citrate	-	+	-	-
Nitrate reduction	+	+	+	+
Urease	-	+	-	-
Indole test	+	-	-	-
Sugar fermentation test				
Arabinose	+	-	-	-
Fructose	-	+	+	+
Glucose	+	+	+	-
Lactose	+	+	-	+
Maltose	-	+	-	+
Mannitol	+	+	-	+
Raffinose	-	-	-	-
Sucrose	+	+	-	-
Xylose	+	-	-	+
Sorbitol	+	-	+	+

+ = positive and - = negative

Molecular identification of isolates

After the morphological and biochemical identification, the isolates were identified by their molecular characterization. The electropherogram of isolates is shown in Figures 3 to 6.

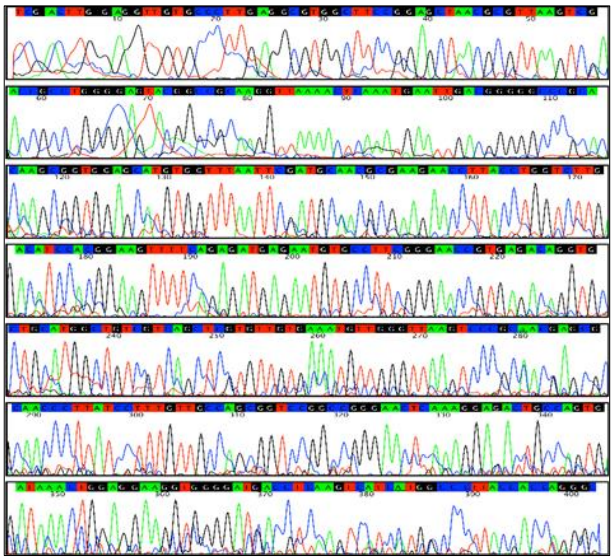

Fig 3: Electropherogram of *Escherichia coli*

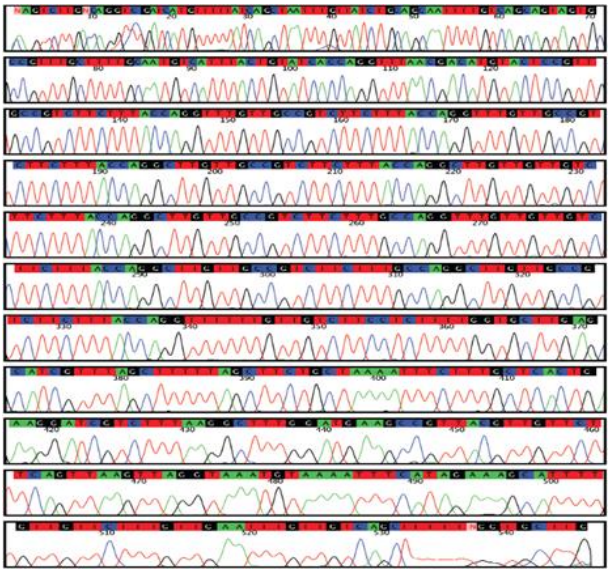

Fig 4: Electropherogram of *Staphylococcus aureus*

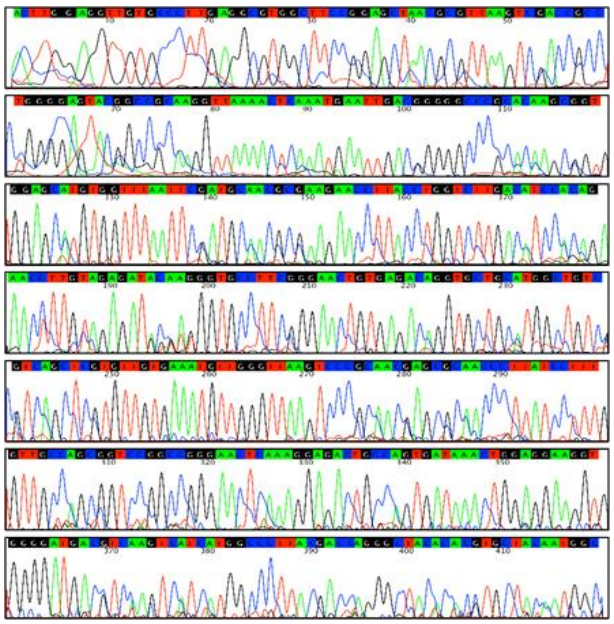

Fig 5: Electropherogram of *Shigella dysenteriae*

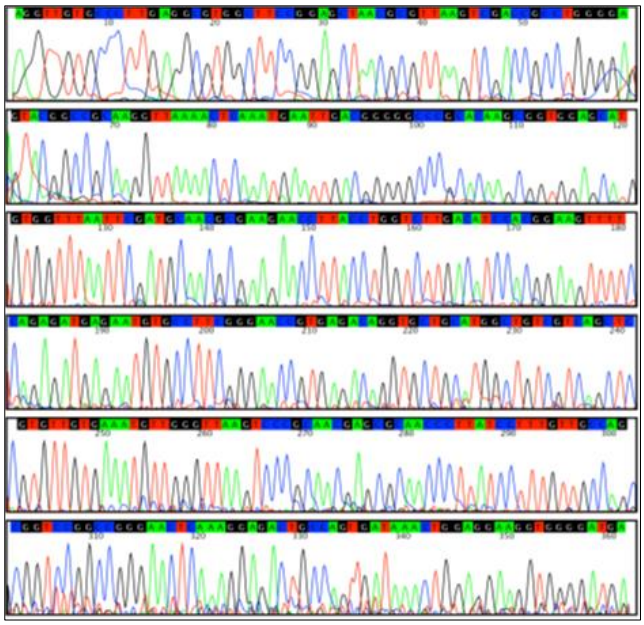

Fig 6: Electropherogram of *Salmonella typhi*

Conclusion

In the present study, a total number of 191 foodborne bacterial pathogens were isolated from selected food samples. The isolates were identified as *Escherichia coli*, *Staphylococcus aureus*, *Shigella dysenteriae*, and *Salmonella typhi* by morphological, biochemical, and molecular characterization. The presence of these pathogens in food samples is an indication of alarming public health concerns. Thus, further study is required to detect the toxic or virulence gene and antibiotic-resistant pattern of these isolates and identify the source of contamination.

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