HIGH SCHOOL EDITION
Journal of Student Research
Volume 13 Issue 4 (2024)

Assessing Microbial Quality of Mumbai's Coastal Waters for Potential Public Health Risks

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ABSTRACT

This study focuses on the statistical estimation of coliform count and isolation of the faecal indicator coliform *Escherichia coli (E. coli)* in water samples collected from 7 beaches along the Mumbai coastline: Aksa, Dana Pani, Versova, Juhu, Bandra, Dadar Chowpatty, and Girgaon Chowpatty. The salinity of seawater was calculated using a pycnometer. The extent of faecal contamination was detected by determining the Most Probable Number in the fresh sample on day 0, and with 7 days intervals up to 21 days for the stored sample. This was done to monitor the coliform count over time. The MPN determination was carried out using MacConkey broth with inverted Durham tubes. Following enumeration by the MPN technique, the representative positive tubes were used to isolate the faecal indicator coliform *Escherichia coli* using MacConkey agar and Eosin-Methylene Blue agar. The typical pink colonies of *Escherichia coli* isolated on MacConkey agar were subjected to biochemical tests such as Indole production, Methyl Red test, Voges-Proskauer test, Citrate utilization Urea hydrolysis, Lactose utilization and the Triple Sugar Iron test. The presence of *Escherichia coli* was further confirmed using Modified MUG EC broth.

Introduction

Mumbai coastline is 149 km long and has a 16 km long beachfront that stretches from Colaba in the South to Marve in the North (Murali, 2021).These beaches are a hotspot for recreational and occupational activities and attract over 1 lakh visitors daily (Bhalerao, 2017). However, the escalating urbanization and industrialization adjacent to these coastal areas have increased the deterioration of the water quality, imposing a potentially significant public health concern. Approximately, 2,700 – 3,000 million litres of sewage is produced in Mumbai every day, of which 25% is released untreated into the city's coastal waters (Bharucha, 2022). Currently in Mumbai there are seven sewage collection zones: Worli, Bandra, Colaba, Versova, Malad, Ghatkopar, and Bhandup. The Sewage treatment plants (STPs) in Worli, Bandra, and Malad are limited to primary treatment, which involves the removal of floating or settled solid particles. Secondary treatment, which biologically degrades organic matter in sewage using oxygen and microbes, is carried out only at the plants in Versova, Bhandup, and Ghatkopar (Virani, 2022). Until 2023, none of these plants were capable of tertiary treatment, which involves chemical purification, but new STPs installed by the government have since enabled this advanced process (Chitnis, 2023). This discharge of inadequately treated household sewage into the coastal waters is a major threat, which significantly increases the levels of organic matter in the natural water bodies and serves as a fertile habitat for the proliferation of various microorganisms, including pathogenic bacteria. One such concerning bacterium is *Escherichia coli*, a faecal indicator coliform, whose presence in natural water bodies directly indicates the extent of faecal contamination which can be used as a measure to confirm the presence of potential faecal pathogens.

*Escherichia coli i*s an omnipresent microorganism and is also found in the gastrointestinal tracts of mammals. While *Escherichia coli* is commonly non - pathogenic, certain strains have pathogenic characteristics. Currently the six types of pathogenic *E. coli* that have been acknowledged are Entero-toxigenic *E. coli* (ETEC), Entero-pathogenic *E. coli (*EPEC), Entero-invasive *E. coli* (EIEC), Entero-haemorrhagic *E. coli* (EHEC), Shiga toxin-producing *E. coli* (STEC), Entero-aggregative *E. coli* (EAEC), and Diffusely-adherent *E. coli* (DAEC) (Maloo *et al*., 2017). They possess virulent properties allowing them to cause a wide range of infections that threaten the health of humans. Since these beaches are common sites for recreational activities, the likelihood of contact between the pathogenic strains and the human body increases. If these bacteria were to infiltrate the human body, they could induce health concerns such as bloody diarrhoea, urinary tract infections, gastrointestinal tract infections, infections of the central nervous system- meningitis and pneumonia (Collier, 2023). Considering the devastating impact that pathogenic *Escherichia coli* can have on human health, it is imperative to study the extent of their presence along the Mumbai Coastline.

Methods

Sample Collection

The study focuses on the extent of faecal pollution of sea water from the Girgaon Beach in the southwest, Dadar in the central region, Juhu, Versova, Dana Pani and Aksa in the northwest region. (Table.1). One litre of water samples was collected in UV sterilized plastic bottles, commencing from Aksa and concluding at Girgaon Chowpatty. The water samples were collected at ankle-level depth, representing the point at which most individuals typically interact with the waters.

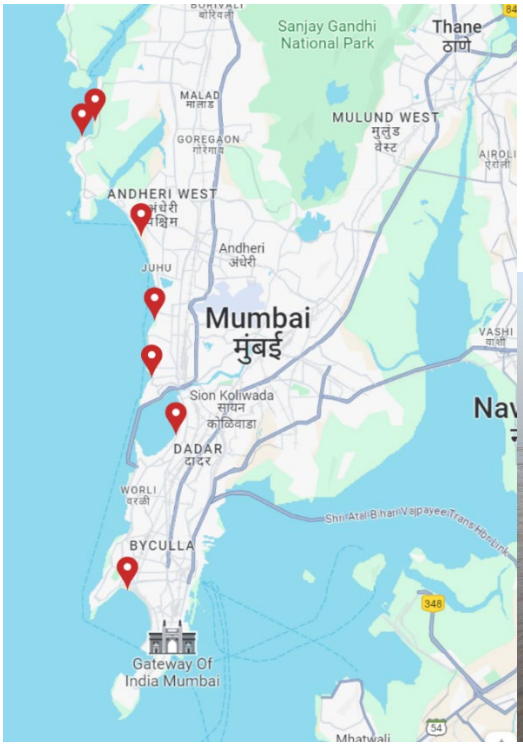

Figure 1. Map of Mumbai city depicting the 7 sample collection points.

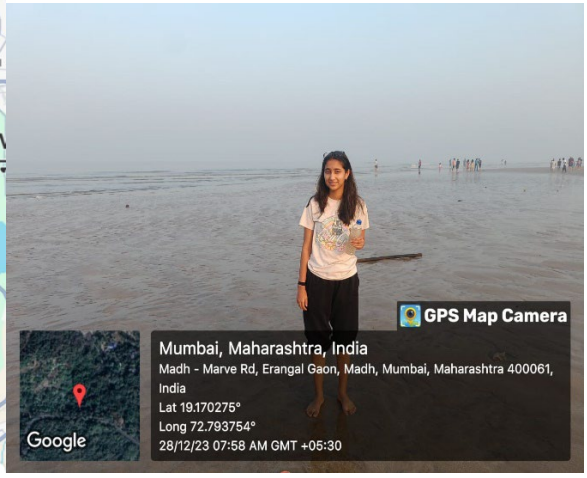

Figure 2. Sample collection.

Table 1. Coordinates of the 7 beaches and a brief description of the area surrounding them.

Beaches	Coordinates	Surrounding Area
AKSA	Lat- 19.175° Long- 72.794°	Popular tourist destination. Spot for recreational water activities and eateries. The area is populated with slums and under-developed residential buildings.
DANA PANI	Lat- 19.170 ° Long- 72.793°	Frequently visited but less compared to others. This beach is surrounded by few slums and under-developed residential buildings.
VERSOVA	Lat- 19.127° Long- 72.816°	Popular tourist destination. Spot for water sports, recreational water activities and eateries.
JUHU	Lat- 19.097° Long- 72.826°	Popular tourist destination. Spot for water sports, recreational water activities and eateries. The area surrounding Juhu Beach features a mix of high-end residential buildings, hotels, and social amenities.
BANDRA	Lat- 19.064° Long- 72.822°	Popular tourist destination. Spot for recreational water activities and eateries. The area surrounding Bandra Beach features a mix of high-end residential buildings, hotels, and social amenities.
DADAR	Lat- 19.027° Long- 72.835°	Popular tourist destination. Spot for recreational water activities and eateries. The area surrounding Dadar Beach features a mix of high-end residential buildings, hotels, and social amenities.
GIRGAON	Lat- 18.954° Long- 72.813°	Popular tourist destination. Spot for recreational water activities and eateries. The area surrounding Girgaon Beach features a mix of high-end residential buildings, hotels, and social amenities.

Most Probable Number

The enumeration of coliforms present in the samples was determined by the Most Probable Number test (Lipps *et al.*, 2023). The samples were inoculated in tubes containing double strength (5 tubes) and single strength (10 tubes) MacConkey Broth Purple with Bromocresol Purple (HiMedia Laboratories, India) with inverted Durham tubes. This broth was used due to its selective and differential nature towards coliforms. The process involved inoculating 10ml of sample into 5 tubes of double strength medium, 1ml of sample into 5 tubes of single strength medium and 0.1ml of sample into 5 tubes of single strength medium and incubating them at 37°C, for 24hrs. The sea water samples were filtered through a filter paper (GE Healthcare, Lifesciences, Whatman Grade-1) and were used for salinity calculations using a pycnometer (J-SIL, 25ml). For the microbiological analysis of

total coliform count in the samples over a period of 21 days, the sea water samples were stored in sealed bottles and maintained at room temperature. The analysis was carried out at 7-day intervals using the previously mentioned Most Probable Number method.

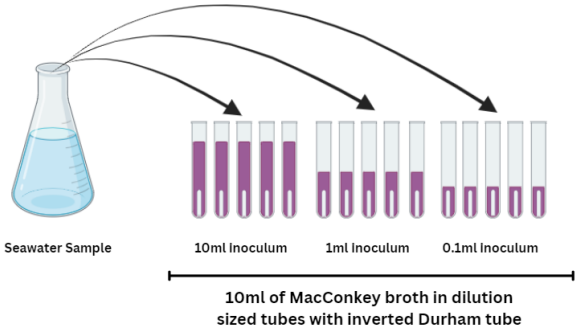

Figure 3. Inoculation of sea water into MPN sets. Created with BioRender.com

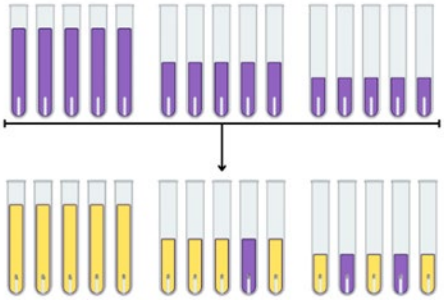

Figure 4. MPN results after 24hr incubation at 37°C. Created with BioRender.com

Key (Figure 4.)

Positive - Yellow colour of medium (Acidic end products) with a bubble in the inverted Durham tube (gas formation).

Negative - Yellow colour of medium (Acidic end products) with no bubble in the inverted Durham tube (no gas formation).

Negative - Purple colour of medium (Alkaline end products) with no bubble in the inverted Durham tube (no gas formation).

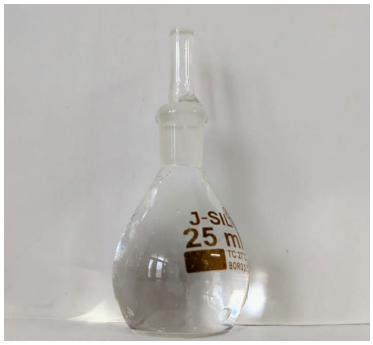

Figure 5. Pycnometer.

Isolation on Plate Media

One representative positive tube from each MPN set (10ml, 1ml, 0.1ml) was used for isolation of coliform bacteria on MacConkey (MAC) agar with 0.15% Bile salts, Crystal Violet & NaCl (HiMedia Laboratories, India) plates and Eosin-Methylene Blue (EMB) agar plates, which were then incubated at 37°C for 24hrs.

Biochemical Identification

The typical isolated colonies from the plate media were used for performing standard biochemical tests specific for *Escherichia coli* - Indole production, Methyl Red test, Voges-Proskauer test, Citrate utilization (IMViC), Urea hydrolysis, Lactose utilization and the Triple Sugar Iron test (Cheeptham and Lai, 2012). Inoculation in Modified MUG (4-Methylumbelliferyl-β-D-Glucuronide) EC broth (HiMedia Laboratories, India) was used as a confirmatory test for *Escherichia coli.*

Results

The total coliform count for all the sea water samples from the 7 beaches (Aksa, Dana Pani, Versova, Juhu, Bandra, Dadar, Girgaon) was calculated to be ≥ 2400 MPN Index/100ml (Table.2), according to the McCrady's probability table (Lipps *et al*., 2022) and United States Environmental Protection Agency (EPA) MPN Calculator. Over the period of 21 days a steady decline was observed in the count of total coliforms in the stored water samples. (Figure 6.)

Table 2. Total coliform counts of the sea water samples over a period of 21 days.

Beaches	**MPN Index/100ml**				
	DAY 0	**DAY 7**	**DAY 14**	**DAY 21**	**% Reduction after Day 21**
AKSA	≥ 2400	23	23	17	99.29 %
DANA PANI	≥ 2400	33	13	11	99.54 %
VERSOVA	≥ 2400	84	49	17	99.29 %
JUHU	≥ 2400	27	23	13	99.45 %
BANDRA	≥ 2400	11	2	2	99.91 %
DADAR	≥ 2400	70	33	22	99.08 %
GIRGAON	≥ 2400	7	2	2	99.91 %

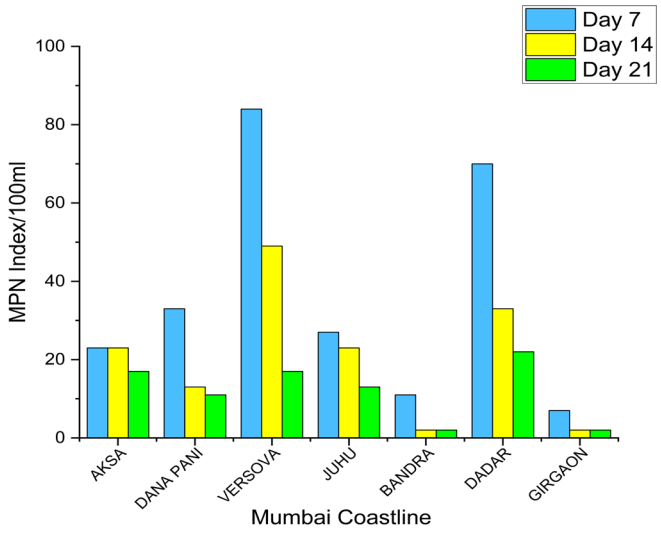

Figure 6. Graph of the microbiological analysis of total coliform count from sea water samples along the Mumbai coastline over a period of 21 days.

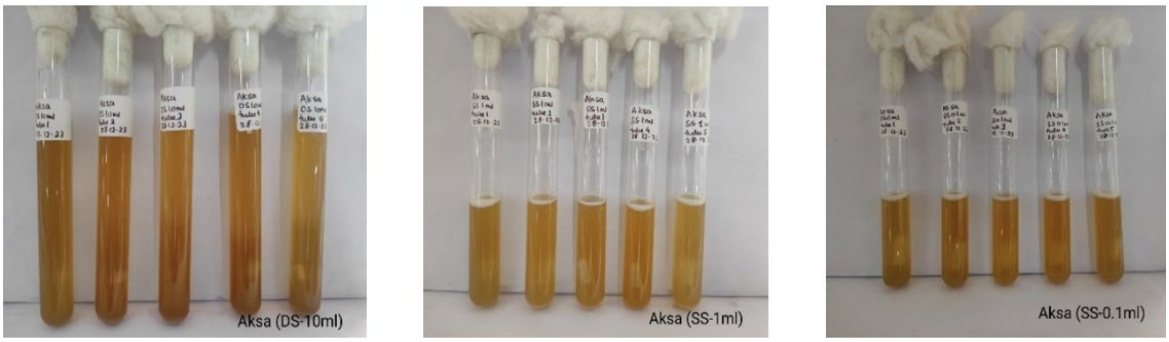

Figure 7. From left to right, positive MPN sets (10ml, 1ml, 0.1ml – Inoculum volume)

The salinity of the seawater from all the beaches (Aksa, Dana Pani, Versova, Juhu, Bandra, Dadar chowpatty, Girgaon chowpatty) was observed to be ranging between 2.3% - 2.6% (Table.3).

Table 3. Salinity levels of the seawater samples.

Beaches	Salinity
AKSA	2.32%
DANA PANI	2.51%
VERSOVA	2.60%
JUHU	2.31%
BANDRA	2.47%
DADAR	2.51%
GIRGAON	2.48%

Pink colonies with a zone of bile precipitation were observed on MAC agar plates and colonies with green metallic sheen were observed on EMB agar plates. 44 isolates form MacConkey agar when characterised

using IMViC, Urea hydrolysis, Lactose utilization, and the Triple Sugar Iron test, resulted in 27 isolates exhibiting standard *Escherichia coli* characteristics (61.36%). From the 27 isolates, 18 (66.66%) were found to possess the β-glucuronidase enzyme using the Modified MUG Test, confirming them as *Escherichia coli*.

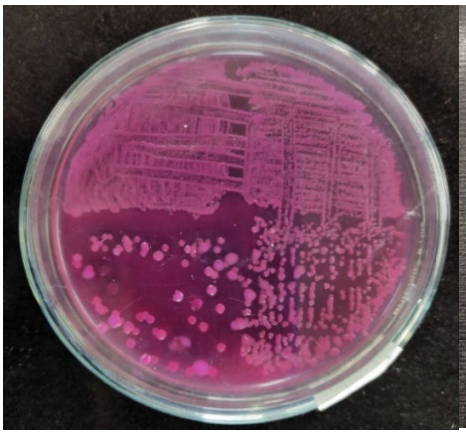

Figure 8. *Escherichia coli* colonies pink colour and zone of bile precipitation on MacConkey agar.

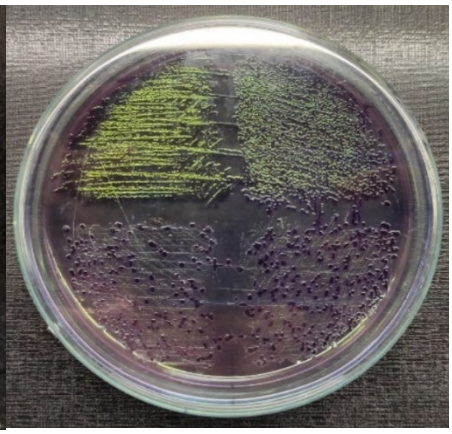

Figure 9. *Escherichia coli* colonies having green metallic sheen on Eosin-Methylene Blue agar

Figure 10. Characteristic biochemical test results for *Escherichia.coli*

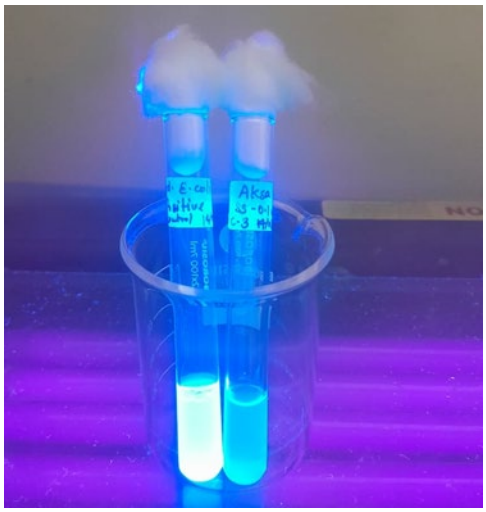

Figure 11. From left to right – positive. MUG result, negative MUG result.

Table 4. Biochemical characterisation of the isolates.

BEACH	*ISOLATE*	*INDOLE*	*MR*	*VP*	*CITRATASE*	*UREA*	*LACTOSE*	*TSI*			*MUG*
								Slant	*Butt*	*Gas*	
Standard	***E. coli***	**+**	**+**	**-**	**-**	**-**	⊕	**Y**	**Y**	**+**	**+**
AKSA	AK-1	+	+	-	+	+	⊕	Y	Y	+	+
AKSA	AK-2	+	+	-	-	-	⊕	Y	Y	+	-
AKSA	AK-3	+	+	-	-	-	⊕	Y	Y	+	-
DANA PANI	DP-1	+	+	-	+	-	⊕	Y	Y	+	+
DANA PANI	DP-2	+	+	-	+	-	⊕	Y	Y	+	+
DANA PANI	DP-3	+	+	-	+	-	⊕	Y	Y	+	+
DANA PANI	DP-4	+	+	-	+	-	⊕	Y	Y	+	+
DANA PANI	DP-5	+	+	-	+	-	⊕	Y	Y	+	+
DANA PANI	DP-6	-	+	-	+	-	⊕	Y	Y	+	-
DANA PANI	DP-7	+	+	-	+	-	⊕	Y	Y	+	+
DANA PANI	DP-8	+	+	-	+	-	⊕	Y	Y	+	+
VERSOVA	V-1	+	+	-	-	-	⊕	Y	Y	+	+
VERSOVA	V-2	+	+	-	-	-	⊕	Y	Y	+	+
VERSOVA	V-3	-	+	+	+	-	⊕	Y	Y	+	+
VERSOVA	V-4	-	+	-	+	-	⊕	Y	Y	+	+
VERSOVA	V-5	-	+	+	+	-	⊕	Y	Y	+	-
VERSOVA	V-6	+	+	-	+	-	⊕	Y	Y	+	+
VERSOVA	V-7	-	+	+	+	-	⊕	Y	P	+	-
JUHU	J-1	-	+	-	+	+	⊕	Y	Y	+	-
JUHU	J-2	-	+	+	+	-	⊕	Y	Y	+	-
JUHU	J-3	-	+	+	+	-	⊕	Y	P	+	-
JUHU	J-4	+	+	-	+	-	⊕	Y	Y	+	+
JUHU	J-5	-	+	-	-	-	⊕	Y	Y	+	+
JUHU	J-6	-	+	-	+	+	⊕	Y	Y	+	-
BANDRA	B-1	+	+	-	-	-	⊕	Y	Y	+	+
BANDRA	B-2	-	+		-	-	⊕	Y	Y	+	+

BANDRA	B-3	+	+	-	-	+	⊕	Y	Y	+	+
BANDRA	B-4	+	+	-	-	-	⊕	Y	Y	+	-
BANDRA	B-5	+	+	-	-	-	⊕	Y	Y	+	-
BANDRA	B-6	+	+	-	+	-	⊕	Y	Y	+	-
DADAR	D-1	+	+	-	-	-	⊕	Y	Y	+	+
DADAR	D-2	+	+	-	-	-	⊕	Y	Y	+	+
DADAR	D-3	-	+	-	-	-	⊕	Y	Y	+	-
DADAR	D-4	+	+	-	-	-	⊕	Y	Y	+	+
DADAR	D-5	+	+	-	-	-	⊕	Y	Y	+	+
DADAR	D-6	-	+	+	+	-	⊕	Y	P	+	+
DADAR	D-7	-	+	+	+	-	⊕	Y	Y	+	-
GIRGAON	G-1	+	+	-	-	-	⊕	Y	Y	+	+
GIRGAON	G-2	+	+	-	-	-	⊕	Y	Y	+	+
GIRGAON	G-3	-	+	-	+	+	⊕	Y	Y	+	-
GIRGAON	G-4	-	+	-	+	+	⊕	Y	Y	+	-
GIRGAON	G-5	-	+	+	+	-	⊕	Y	P	+	-
GIRGAON	G-6	-	+	+	+	-	⊕	Y	P	+	-
GIRGAON	G-7	+	+	-	-	-	⊕	Y	P	+	-
GIRGAON	G-8	+	+	-	-	-	⊕	Y	Y	+	-

KEY
+ Enzyme activity detected.
- No Enzyme activity detected.
For Lactose utilization-
⊕ Acid with gas.
For Triple sugar iron test-
Y Yellow
P Pink
+ Gas.

Discussion

The total coliform count across all seven beaches exceeded 2400 MPN Index/100ml, in spite of slight variation in salinity of the sea waters. This indicated that the entire stretch of the Mumbai coastline is heavily contaminated, likely due to the discharge of inadequately treated sewage into the Arabian Sea. Release of such sewage becomes an escalating threat to the marine life and the health of the community using beaches for recreational purposes. A study conducted by the CSIR-NIO (Fulke *et al.*, 2024) on two of the same beaches- Aksa, and Dana Pani, the highest coliform count was 1600 MPN Index/100ml. Furthermore, the data from this study aligns with a similar study done in 2017 (Maloo *et al.*, 2017). This indicates that the microbiological quality of the Mumbai coastal waters has significantly deteriorated over the past 7 years.

The environmental standard established by the Central Pollution Control Board (CPCB) in 1998, classifies SW-II waters, intended for activities such as contact water sports, bathing, and commercial fishing, with a safety limit for the total coliform count as 100 MPN Index/100 ml (G.S.R 7, dated Dec. 22, 1998). The results of this study are over 24 times higher than the prescribed limit, showing the severely compromised sewage treatment process and hazardous nature of Mumbai's coastline. Additionally, the CPCB guideline states that the coliform count of potable water should be lower than 50 MPN Index/100 ml. During recreational activities, water ingress into the body is inevitable, and the significantly elevated coliform counts observed in these waters' present a substantial risk to human health, potentially leading to various infections.

The microbiological analysis for total coliform count of the stored samples carried out at 7-day intervals, revealed a general decline in the MPN index of the coliforms over 21days. During these 21 days, the water samples were stored in clean, sterile, and sealed plastic bottles at room temperature. The load of *E. coli* in the samples was probably decreased due to the depletion of nutrients (both organic and inorganic), pH, and dissolved O_2 (Rozen & Belkin, 2005). Organic carbon and energy sources are essential for the basic metabolic processes of *Escherichia coli*; thus, a decrease in the carbon concentration can inhibit the production of ATP and biosynthetic precursors that are essential for growth (Bren *et al*., 2016). Over these 21 days, none of the stored samples showed a complete inhibition of *Escherichia coli*. This indicates that, even in the absence of sufficient nutrients, *Escherichia coli* can persist at low concentrations. This resilient nature underscores the ability of *Escherichia coli* cells to flourish in coastal waters, where continuous sewage discharge supplies the necessary nutrients for its growth. A similar study in the coastal regions of Puerto Rico demonstrated a significant decline in *Escherichia coli* concentrations immediately after the stop of effluent discharge, showcasing the importance of organic nutrients for their survival (Lopez-Torres *et al*., 1988).

Isolates that showed a positive MUG result demonstrating fluorescence under UV can be considered as *Escherichia coli* when corroborated by additional test results i.e. pink colonies on MAC agar plates, colonies with a green metallic sheen on EMB agar plates, and biochemical results characteristic of standard *Escherichia coli.*

However, there were isolates that displayed all the characteristics typical of standard *Escherichia coli* but did not possess the β-glucuronidase enzyme activity. Such isolates could possibly be *Escherichia coli* O157:H7 strains. This is because O157:H7 strains lack the enzyme β-glucuronidase, resulting in a negative MUG test. These isolates are needed to be further identified using immunological methods to be other Entero-toxigenic *Escherichia coli* (ETEC), Entero-pathogenic *E. coli (*EPEC), Entero-invasive *E. coli* (EIEC), Entero-haemorrhagic *E. coli* (EHEC), Shiga toxin-producing *E. coli* (STEC), Entero-aggregative *E. coli* (EAEC), and Diffusely-adherent *E. coli* (DAEC).

Conclusion

This study monitors the microbiological quality of seawater along the Mumbai coastline, with respect to faecal coliforms and confirms the presence of *Escherichia coli.* The 7 beaches studied had a widespread presence of faecal contamination because of the constant exposure to poorly treated sewage discharge. The findings reveal that the Most Probable Number of Faecal coliforms was 24 times higher than the limit set by the Central Pollution Control Board, highlighting the deteriorated quality of the coastal waters. Therefore, these beaches pose a significant public health risk to frequent visitors especially children, senior citizens, and people with open wounds, exposed to the contaminated waters. This necessitates urgent action from government authorities regarding sewage management and sanitation facilities. The data from this study can aid officials in establishing a comprehensive monitoring system that emphasizes environmental planning and the management of coastal areas. Urban planning is another method, that involves integrating sustainable practices to manage domestic sewage and waste effectively. The new infrastructural developments should ensure that the quality of coastal waters is maintained as per the environmental standards to prevent potential outbreaks of water-borne infections in the future.

Limitations

The initial idea for this research revolved around creating awareness in society on the recent deterioration of the sea water quality along the Mumbai coastline and the possibility of it becoming a point of origin for a plethora of outbreaks caused by medically important infectious pathotypes of *Escherichia coli.* This included screening for a large subset of *Escherichia coli* isolates from Mumbai seawater and identifying them as various

pathotypes using serological and genomic techniques. This idea faced quite a few challenges. The lack of modern infrastructure and limited funding did not permit the initial objectives of this research to be fulfilled. These constraints restricted the broader and extensive scope of the research and narrowed it down to the objectives of this paper. With better facilities and funding, this project could achieve outcomes that could draw the attention of civic authorities, for promoting corrective measures to address this critical public health issue.

Acknowledgments

I would like to thank Dr. Radhika Birmole for her insightful guidance in designing and execution of this project. I would also like to thank Dr. Anuradha Pendse, Head of Department, Microbiology, Wilson College Autonomous for allowing me to conduct my research in their laboratory and providing me with the necessary facilities. I am thankful to Parth Arolkar and Siddhi Sawant, as mentors for developing experimental skills for completion of this project. Lastly, I would like to thank the Principal Prof. Anna Pratima Nikalje and Wilson College management for permitting me to complete my project.

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