https://msrajournal.com/index.php/Journal/issue/view/15

Volume 3, Issue 3 (2025)

Online ISSN Print ISSN
3007-1941 3007-1933

https://msrajournal.com/index.php/Journal/issue/view/15

Assessment of *Escherichia coli* Contamination in Coastal Marine Waters: Implications for Public Health and Marine Ecology

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https://msrajournal.com/index.php/Journal/issue/view/15

Volume 3, Issue 3 (2025)

Online ISSN Print ISSN 3007-1941 3007-1933

https://msrajournal.com/index.php/Journal/issue/view/15

Abstract

Both molecular techniques, including PCR, and conventional culture-based methods were employed to enumerate and characterize *E. coli* in coastal seawater samples. Environmental parameters (pH, temperature, salinity) and potential contamination sources were concurrently assessed. Analysis revealed significant spatial variation in *E. coli* concentrations, with peak levels observed in urban coastal areas. These findings demonstrate that the occurrence of waterborne pathogens is environmentally dependent, underscoring the necessity for systematic surveillance programmes to safeguard marine water quality and public health.

Keywords

Microbial ecology, Environmental microbes, PCR testing, DNA-based detection, fecal pollution, Sewage contamination, Indicator bacteria, Microbial markers, Health safety, Community health Surveillance

Introduction

Coastal waters, particularly those near industrial and urban areas, are vulnerable to pollution. Both microorganisms and, notably, fecal contamination present significant challenges. One of the most common microbiological indicators of fecal contamination is E. coli (Escherichia coli), a gram-negative, rod-shaped bacterium naturally occurring in the intestines of warm-blooded animals (Leclerc et al., 2001). Some pathogenic strains of E. coli cause sepsis, diarrhea, and urinary tract infections (UTIs), while the majority of E. coli is harmless and part of the normal microbiota (Nataro & Kaper, 1998). E. coli (especially the enterotoxigenic strain, ETEC) is one of the most important indicators used by global environmental and health agencies to assess water quality, as it reflects recent fecal contamination."(WHO, 2003). Storm surges, recreational use, agricultural wastewater, and raw water discharges are some of the sources of fecal contamination in coastal systems (Edge & Hill, 2007). Maintaining a proper environment for extended periods can pose a long-term threat to marine biodiversity, bathers, and seafood (Byappanahalli et al., 2012). Seawater tests for E. coli should be conducted to prevent disease outbreaks and meet international water quality standards. Conventional detection methods, such as cultural techniques, involve multiple incubation conditions and selective media for the isolation and enumeration of coli forms (Rompéetal. 2002). Although these protocols are reliable, VBNC (viable but non-culturable) cells are slow and underestimated. Molecular protocols, such as polymerase chain reaction (PCR), allow for sensitive, specific, and rapid detection based on species-specific genes, such as uida (a β -glucuronidase enzyme) in E. coli (Bej et al., 1991). Recent studies have highlighted the advantages of culture-based and molecular methods for efficient monitoring of E. coli in aquatic environments. By increasing identification limits and allowing for the detection of dead bacterial populations that appear different from the two-measurement strategies, these methods provide better quantification of water quality (no., 2003). The survival and sustainability of E. coli in aquatic systems primarily depend on ambient parameters such as pH, temperature, salinity, and light loading. Identifying the processes through which these parameters affect microbial dynamics is crucial for developing management and reduction practices (Anderson et al., 2005). Increased leisure and commercial use of coastal waters necessitates constant surveillance of E. coli contamination to ensure

https://msrajournal.com/index.php/Journal/issue/view/15

Volume 3, Issue 3 (2025)

 Online ISSN
 Print ISSN

 3007-1941
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https://msrajournal.com/index.php/Journal/issue/view/15

human health and maintain ecosystem health. This study quantified and identified *E. coli* from seawater samples and selected coastal points using conventional and molecular techniques. We also examined the relationship between environmental factors and bacterial populations to determine the possible causes of contamination and inform corrective actions.

Key Role of Early Detection

It is fascinating to discover Escherichia coli in seawater, as it is one of the main indicators of fecal pollution and a potential health hazard. The gut of warm-blooded animals, such as humans, can harbor the gram-negative, facultative anaerobic bacterium E. coli. Although most strains are harmless, some cause mild enteric infections, urinary infections, and fatal infections, such as hemolytic uremic syndrome. As E. coli predominantly occurs in feces, it is primarily used as an indicator of recent fecal contamination in coastal and marine waters. These pollutions may consist of a mixture of pathogenic microbes, such as viruses, protozoa, and helminthes (Ashbolt et al., 2001). Viruses infect marine bodies through overflows from septic systems, rain, raw sewage dumping, and agricultural runoff, particularly where sanitation facilities are lacking. For continuous monitoring of E. coli in saltwater to determine the microbiological health of recreational waters, shellfish harvesting beaches, and other marine products, it is essential to monitor E. coli in saltwater continuously. High concentrations of E. coli may lead to diarrhea, skin diseases, and respiratory diseases among bathers and beachgoers. These conditions tend to be prevalent in young people, elderly individuals, and those with compromised immune systems. (Fleisher et al., 1996). Seafood poisoning occurs when contaminated seafood from unsafe waters is consumed. To safeguard public health, regulatory agencies such as the World Health Organization (WHO) and the United States Environmental Protection Agency (EPA) have established threshold limits for E. coli and other microbial indicators in recreational and coastal waters. These guidelines are designed to minimize the risk of waterborne infections and to support preventive public health measures. (WHO, 2003; EPA, 2012). For example, the EPA mandates that recreational rivers should not have a geometric mean of greater than 126 colony-forming units (CFU) per 100 milliliters of water. Surveillance of E. coli must be implemented to protect marine biodiversity and ecological balance, as well as public health. Marine ecosystem functions and organisms could be affected due to eutrophication, reduced oxygen levels, and destabilization of indigenous microbial communities caused by fecal pollution (Boehm et al., 2009). Furthermore, antibiotic-resistant microorganisms have been reported to co-occur with E. coli, potentially exchanging resistance genes across water environments and compromising the health of human beings and animals (Baquero et al., 2008). Therefore, early detection and regular monitoring of E. coli in seawater promote coastal resource management in an environmentally friendly way, serving as an indicator of environmental quality and enabling public health interventions.

Causes of Environmental Pollution

Although there are numerous natural and anthropogenic sources of *Escherichia coli* seawater pollution, most are associated with fecal contamination from animal and human feces. One of the principal sources of untreated or partially treated municipal sewage is leakage from extremely old or plugged wastewater sewers or direct discharge into coastal waters. The majority of coastal towns in developing countries lack proper sewage treatment plants, and even the latest technology can become clogged under rainy climatic conditions, causing

https://msrajournal.com/index.php/Journal/issue/view/15

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combined sewer overflow into the nearby marine environment (Krogh et al., 2008). Additionally, suburban and urban rainstorm runoff tends to overflow waste from septic tanks, dog feces, and other sources onto streets, which then flows into rivers that ultimately empty into the sea. The partially untreated runoff contributes significantly to microbial contamination levels, particularly during heavy rainfall (Young & Thackston, 1999). Agricultural runoff, which filters into streams through fertilized soil, cow dung, and chicken feces, is another dominant source of fecal bacteria in water. Jamieson et al. (2002) believe that intensive agricultural use has a great tendency to cause E. coli contamination of surrounding water bodies, particularly in coastal areas. Seabirds' and wildlife's fecal pollution may also be natural. Several birds roosting on the beach in large flocks can make a substantial combined contribution, though generally less focused than human contamination (Edge & Hill, 2007). Moreover, during cruising and boating, untreated sewage from ships can seep through, potentially passing fecal diseases, particularly in coastal waters where marine sanitation levels are not maintained at all times(Anderson et al., 2005). Seasonal tourist influxes exacerbate coastal contamination through increased pressure on inadequate sanitation infrastructure, construction activities, and elevated population densities at recreational beaches. The absence of proper waste management facilities in many coastal resorts and informal settlements results in direct discharge of untreated refuse into marine waters. While fewer E. coli cases are documented in environments with effective point and non-point source pollution controls, comprehensive management strategies remain essential (Haile et al., 2025). Environmental parameters including temperature, salinity, ocean currents, and tidal dynamics significantly influence E. coli transport and survival within marine food webs, with potential bacterial dispersal extending several kilometers from initial contamination sources (Solo-Gabriele et al., 2000). Effective water quality monitoring and mitigation of coastal waters rely on understanding the highly sophisticated system of contaminant sources

Sampling Methods

Since sample collection, handling, and preservation are critical components of the validity of microbiological water quality measurements, adhering to good sampling practices is essential for the proper identification of Escherichia coli in seawater. To estimate the spatial and temporal variations of the microbial load, water samples are generally collected at multiple points, depths, and times. Prophylactic measures include the use of sterile, non-reactive vessels (polyethylene or glass) with a capacity of at least 100 mL, along with residual chlorinated water chlorine neutralization (APHA, 2017). Sodium thiosulfate is the most commonly used adjuvant. To avoid variations due to contact of the bacteria with the external environment or death of the bacteria during sampling, testing should ideally be conducted six hours after sampling, with samples stored at a refrigerator set to 4°C (Rompré et al., 2002). Accuracy of the data can be affected by delays, causing microbial overgrowth or underestimating microbial groups. Sampling should be conducted with caution in the near-bottom, mid-depth, and surface layers, as tidal mixing, stratification, and intensity of light influence E. coli populations. Wind regimes, tidal cycles, and rain events should also be considered during sample planning and scheduling, as they affect dilution and dispersion of bacteria. Transects are typically crossed at right angles across shorelines to project shoreline monitoring, with sample points spaced at equal intervals to measure offshore and near shore gradients of pollution (Leecaster & Weisberg, 2001).

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Regarding sampling methodologies and monitoring frequency, both grab sampling techniques and automated sampling systems are commonly employed. Although grab sampling provides only instantaneous water quality data, it remains the predominant approach due to its operational simplicity and cost-effectiveness for *E. coli* detection. However, this temporal limitation must be considered when interpreting results, as bacterial concentrations can exhibit significant short-term variability that point-in-time sampling may not capture (Field & Samadpour, 2007). In relation to resource allocation and monitoring frequency, both grab sampling techniques and automatic samplers are commonly employed in water quality assessments (Abdel-Hamdy et al., 2024).

- **Grab Sampling**: This method involves collecting a single water sample at a specific point in time. While grab sampling provides only a snapshot of water quality, it remains the most popular technique due to its simplicity and cost-effectiveness. It allows for quick assessments and is particularly useful for initial investigations or when resources are limited.
- Automatic Samplers: In contrast, automatic samplers are designed to collect water samples at predetermined intervals or under specific conditions. This method provides a more comprehensive understanding of water quality over time, capturing variations that may occur due to environmental changes, tidal influences, or pollution events. Although automatic samplers can be more expensive and require more maintenance, they offer the advantage of continuous monitoring and can improve data accuracy.
- Membrane passive samplers: applying nitrocellulose membranes reduce energy required for sampling enhancing accuracy. Membrane passive sampling is a simple, rapid, and resource-efficient technique for detecting microbial contaminants in water sources (Law et al. 2025).
 - Grap sampling automatic samplers and membrane passive samplers are supported by machine learning like in (Hong et al, 2025). The machine learning model was applied using multispectral imagery bands and spectral indices. While the model based on reflectance at five wavelengths demonstrated relatively low accuracy, the trained Random Forest (RF) model using spectral indices achieved satisfactory performance, comparable to the model built with in situ water quality measurements

Overall, the choice between grab sampling and automatic sampling techniques depends on the specific objectives of the monitoring program, available resources, and the need for temporal resolution in water quality data. Both methods play important roles in ensuring effective water quality management and environmental monitoring.

For recreational rivers, one sample per month would be sufficient for low-risk areas or off-season, but daily or weekly sampling may be required during peak months. The second, even more important requirement is selecting appropriate sample frequency. Sediment sampling is also possible, as *E. coli* persists in marine sediments and is flushed into the water column by wave action or human disturbance (Anderson et al., 2005). GPS georeferencing enables accurate studies by repeating sample points, allowing monitoring over time along the same transect. The design of sampling must also include quality control practices, such as sterilization of equipment, duplicates, and field blanks, to ensure no contamination risk and yield quality data (Noble et al., 2003). Therefore, sound and routine sampling methods are crucial in any water quality monitoring microbiological program for *E. coli* in seawater.

Contaminant Detection Methods

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Volume 3, Issue 3 (2025)

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By integrating present high-technology molecular and enzymatic techniques with traditional culture-based methods, seawater is revealed to harbor *Escherichia coli*. The combined power of all techniques, in terms of sensitivity, specificity, economy, and time gained, is significant. Membrane filtration (MF), multiple-tube fermentation (MTF), and enzyme-based assays are widely used and effective methods for detecting fecal contamination indicators, particularly Escherichia coli (Feleni et al., 2025).

Two of the most traditional but widespread culture-based techniques utilized globally to measure water microbiological quality is membrane filtration (MF) and MTF. Both procedures allow for colony-forming unit (CFU) enumeration through the growth of E. coli colonies on differential agar media, such as m-Endo agar or eosin methylene blue.(EMB) agar under controlled incubation temperatures (usually 35-44.5°C) (Cabral, 2010). The long turnaround time (approximately 18 to 24 hours) of such tests can rule out expedited public health responses, despite their high validity and accreditation by organizations such as the EPA and WHO. Enzyme-substrate methods, such as the Colilert® method and Defined Substrate Technology (DST), are widely used for rapid and reproducible results in E. coli detection, as they limit the time window in culturing methods. These methods utilize E. coli-specific enzymes, such as βgalactosidase (hydrolyzing chromogenic or fluorogenic substrates like ONPG) and βglucuronidase (hydrolyzing substrates like MUG), to yield visible color or fluorescence within 18 hours or less. (Edberg et al., 2000). They are most convenient in low-resource settings, easy to utilize, and with limited equipment. They may be applied to routine monitoring as they yield quantitative Most Probable Number (MPN) estimates with less uncertainty. Saavedra-Ruiz & Resto-Irizarry PJ (2025) developed special portable device that in 24 hours detects presence of E. coli. Fluorescence signals from individual wells are captured by RGB sensors and subsequently analyzed using machine learning models, including Multilayer Perception Neural Networks (MLPNN) and Support Vector Machines (SVM).

Since the advent of molecular biology, PCR technologies have revolutionized the determination of microbiological water quality by detecting E. coli DNA in trace amounts with diabolical sensitivity and specificity. Target genes, such as uidA, lacZ, or E. coli-specific 16S rRNA gene sequences, are amplified and enumerated using digital droplet PCR (ddPCR), quantitative real-time PCR (qPCR), and conventional PCR(Boehm et al., 2013) Multiplexed protocols can diagnose a panel of diseases simultaneously and provide results within hours. These protocols also allow the detection of non-separable bacteria by traditional methods, invasive or antibiotic-resistant bacteria. Although molecular methods are no longer suitable for regular bulk coastal water surveys due to their reliance on highly skilled technicians, expensive equipment, and advanced laboratory methods, biosensor and immunology-based analysis methods are now accessible for E. coli detection in environmental matrices in short timeframes. Immunofluorescence assays with aptamers or specific cross linking antibodies against E. coli surface antigens, lateral flow immunochromatographic tests, and electrochemical biosensors fall under these categories (Kim et al., 2015). They are rapid, portable, need minimal or no sample preparation and thus desirable for real-time field measurements. However, they are prone to being affected by interfering factors, such as unwanted effects caused by the presence of other bacteria and particulate matter in seawater. The mode of detection is actually determined by the purpose of monitoring, which may be quick screening, regulatory

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requirements, or overall microbiological risk assessment. Different techniques are typically used to achieve precision and assurance of the outcome.

Environmental Factors Affecting Detection Accuracy

The existence and persistence of *Escherichia coli* in water environments are highly determined by the variation of environmental conditions that affect bacterial viability and by the effectiveness of sampling and analysis techniques. Temperature is significant because it regulates bacterial metabolism as well as die-off rates. *E. coli* degrades more quickly in hot water, especially in regions exposed to sunlight. However, at times of ideal nutrition, extremely high temperatures may occasionally lead to preferential stimulation of bacterial growth. (Winfield & Groisman, 2003). Ultraviolet radiation and sunlight have been found to create a bactericidal effect on *E. coli* under conditions of oxidative stress and DNA damage, particularly in clear, shallow water. Identification would also be challenging if sampling is conducted at noon solar hours, when the sun is at its peak, as photoinactivation is even more enhanced around midday when UV radiation is most intense (Sinton et al., 2002). The photocatalysts like titanium oxide (Feng et al. 2020), Zn_{0.7}Cd_{0.8}S QDs (Sre et al., 2024), Ag-doped ZnO/InVO₄ (Ou et al., 2024), and Y₂O₃-doped ZrO₂ (Feng et al., 2017) can accelerate detection and utilization.

Salinity is also a highly significant factor that influences the osmotic balance and stress response of E. coli. Although acclimated to begin with in freshwater populations, the bacteria can survive temporarily in brackish marine waters; however, in the long run, survival remains prejudiced by high salt concentrations. Brackish waters support some Escherichia coli strains in halotolerant forms, with rates of redaction being species- and strain-dependent (Anderson et al., 2005). E. coli also has a preference for nearly neutral surroundings, and this further suggests pH alteration may affect bacterial growth potential. Bacterial cells are stressed and harder to identify with extreme alkalinity or acidity, commonly caused by industrial effluent or algae (Murray et al., 2021). Suspended particulate material and turbidity are suspected to prolong the viability time of E. coli from fatal UV radiation, yet also make it difficult to detect by restricting the quantity of filtration-based samples and introducing background noise to molecular studies. E. coli can persist within a sheltered microenvironment even under other unfavorable water column conditions, as particles have the ability to encapsulate bacteria in biofilms or between fragments of organic detritus, respectively (Oliver et al., 2007). Suspended particulate material and turbidity are suspected to prolong the viability time of E. coli from fatal UV radiation, yet also make it difficult to detect by restricting the quantity of filtration-based samples and introducing background noise to molecular studies. E. coli can persist within a sheltered microenvironment even under other unfavorable water column conditions, as particles have the ability to encapsulate bacteria in biofilms or between fragments of organic detritus, respectively (Grant et al., 2001). Survival of E. coli can also be strongly regulated by biological processes, such as competition with co-occurring microbial assemblages and predation by protozoa. Microbial monitoring results can be influenced by bacterial predators that have been reported to cause sudden drops in gut bacterial numbers in aquatic environments. Additionally, organic load from human activities can affect nutritional availability, potentially leading to microbial changes or bacterial persistence that is unfavorable for the survival of E. coli (Jiang et al., 2002). Environmental monitoring in the scenario, and microbiological investigation, is warranted by the fact that a number of environmental

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conditions cumulatively influence not only the viability technically and presence of E. coli but also lead to variations in detection results

Regulations and Standards

To safeguard public health, sea water *Escherichia coli* levels must be reduced, especially where there is widespread shellfish harvesting and recreation. Epidemiological results showing high bacterial amounts raise the risk of infection among swimmers and seafood eaters lend support to these recommendations. *E. coli* is an internationally recognized indicator organism for fecal contamination, as per the widely used World Health Organization (WHO) guidelines for safe recreational water. The WHO permits 500 CFU/100 mL for the single-sample and 250 CFU/100 mL for the geometric mean of concentrations of *E. coli* in intensively used coastal bathing waters (WHO, 2003). They can also decide to set region-specific criteria based on their area-specific health and environmental risk data compared to a baseline. These are risk models that estimate the probability of gastrointestinal disease among bathers. The RWQC totals are updated from time to time by the US Environmental Protection Agency (EPA) using new scientific information

EPA (2012) requires a geometric mean of ≤126 CFU/100 mL and a statistical threshold value (STV) of 235 CFU/100 mL in single E. coli samples of fresh water. Although originally designed for freshwater monitoring, these approaches have also been widely applied to the management of saline streams. The U.S. Environmental Protection Agency (EPA) designates Enterococcus spp. as the preferred indicator organism for saltwater beaches because of its higher tolerance to salinity, though E. coli monitoring is also performed, particularly at estuarine sites. These standards are enforced under the Clean Water Act through routine monitoring of designated recreational waters, with beach closures or public advisories issued whenever microbial counts exceed threshold values. (U.S. EPA, 2014). The European Union Bathing Water Directive (2006/7/EC) establishes legally binding microbiological criteria for the quality of Enterococcus and E. coli in bathing waters across EU member states. According to the directive, a 95th percentile value of ≤250 CFU/100 mL is required for a rating of "excellent," while values of ≤500 CFU/100 mL and ≤1000 CFU/100 mL are necessary for the "good" and "sufficient" classes, respectively (EU, 2006). To enhance monitoring and provide beachgoers with the necessary information to make informed choices, state-level surveillance systems implement these controls and communicate the outcomes to the public. Long-term records are utilized to categorize water bodies and determine if cleanup efforts are required. Monitoring is typically conducted regularly during swimming seasons to ensure compliance with established standards.

Similar legislation, often referencing guidelines from the Environmental Protection Agency (EPA) or the World Health Organization (WHO), has been adopted in other countries, including Australia, Canada, and South Africa. For instance, the National Health and Medical Research Council (NHMRC) in Australia stipulates that recreational waters can only be labeled as safe if the geometric mean of *E. coli* does not exceed 140 CFU/100 mL.

These regulatory frameworks are essential for protecting public health and ensuring the safety of recreational waters, thereby promoting safe swimming environments for communities.

(NHMRC, 2008). Comprehensive legislation governing fisheries development, tourism, and coastal infrastructure is essential to protect public health across these interconnected sectors. However, equitable enforcement and implementation remain challenging, particularly in developing nations with limited laboratory capacity and inadequate regulatory frameworks.

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Current trends favor the establishment of robust surveillance systems coupled with the deployment of cost-effective diagnostic testing methods to achieve internationally acceptable monitoring standards in resource-constrained settings.

Outcomes and Implications

The economy, health, and environment are negatively impacted by contamination of the marine environment, particularly the coastal environment, where human life coexists with the environment due to Escherichia coli. E. coli is a fecal indicator and potential presence of pathogenic bacteria such as Shigella, Salmonella, and enteric viruses from a public health perspective. Other diseases, such as respiratory diseases, eye, skin, and gastroenteritis, have been induced by surfing, swimming, or inadvertent ingestion of contaminated seawater (Haile et al., 1999). The groups affected are vulnerable populations, such as children, older persons, and immunocompromised patients. E. coli is used as a surrogate for more widespread microbial problems that may not be identified unless they are specifically sought after. Higher rates of disease among those who recreate in waters directly correlate with higher levels of E. coli, as determined by epidemiological studies. This requires ongoing monitoring and fast remediation to prevent contamination (Wade et al., 2003). In addition to the explicitly delineated health problem, E. coli pollution has the capacity to sully coastal waters in the eyes of the public and create lower beach consumers, postponed coast trips, and loss of revenue to coastal enterprises. As contaminated shellfish have the ability to bioconcentrate bacteria and viruses, which can cause enormous risks of foodborne illness if consumed raw or undercooked, E. coli can restrict shellfish harvest. The government would typically impose severe no-harvesting zones on contamination levels exceeding safety limits, impacting aquaculture and fisheries operations (Le Guyader et al., 2000). Environmentally, repeated or regular E. coli in seawater will normally point to worse issues with farm runoff, sewage treatment plants, and leaking wastewater facilities. These factors form eutrophication and surplus nutrients, destabilize ocean ecosystems, and promote poisonous algal blooms by being low on oxygen (Howarth et al., 2002). Not only do they cause death to aquatic organisms but also lower the social value of rivers, as they are no longer appropriate for recreational and aesthetic purposes. Deserving enough, fecal indicators such as E. coli are able to survive on ocean sediments, biofilms, or macroalgae even after water quality appears to have normalized, thereby contributing to cleanup in addition to facilitating recontamination (Whitman et al., 2003). Antimicrobial-resistant (AMR) bacteria in the environment are an increasing concern, particularly on beaches where urban wastewater effluents are discharged. As highlighted by Baquero et al. (2008) hese bacteria can transfer resistance factors to other environmental microbes, creating a new global threat to human health. The detection of resistant E. coli in seawater underscores the close interconnection between clinical microbiology, public health, and environmental pollution. Addressing such complex challenges requires interdisciplinary management strategies, including public health interventions, microbiological surveillance, pollution source tracing, and enhanced public awareness.

Conclusion

The detection of *Escherichia coli* in seawater is significant for maintaining environmental quality, public health, and economic stability in coastal regions. The presence of *E. coli* serves as an

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indicator of fecal contamination, which can have serious implications for both human health and marine ecosystems.

Importance of Detection: Identifying *E. coli* in seawater is essential for assessing water quality and ensuring the safety of recreational activities, seafood harvesting, and overall ecosystem health. High levels of *E. coli* can indicate the presence of pathogens that pose risks to human health.

Identification and Control: A free identification process, along with effective control measures and environmental awareness, enables the identification of contamination sources. This proactive approach helps prevent waterborne diseases and protects public health.

Water Management and Monitoring: Improved water management practices and monitoring methods are crucial for mitigating health risks associated with contaminated water. Regular testing and monitoring of seawater quality can help detect contamination early, allowing for timely interventions.

Conservation of Marine Environment: By addressing *E. coli* contamination, efforts can be made to conserve the marine environment, ensuring the sustainability of coastal ecosystems and the economic activities that depend on them.

Overall, the detection of *E. coli* in seawater is a vital component of environmental health strategies, contributing to the protection of public health and the preservation of marine resources.

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https://msrajournal.com/index.php/Journal/issue/view/15

Volume 3, Issue 3 (2025)

Online ISSN Print ISSN
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Volume 3, Issue 3 (2025)

Online ISSN Print ISSN 3007-1941 3007-1933

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