RESEARCH ARTICLE

Detection of pathogenic microorganisms in ballast water of internationally trafficked vessels that arrived at maritime ports on the Colombian Atlantic coast during the years 2020 to 2023

Detección de microorganismos patógenos en agua de lastre de buques de tráfico internacional que arribaron a puertos marítimos de la costa Atlántica colombiana durante los años 2020 a 2023

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ABSTRACT

Ballast water has historically been used in vessels to maintain their stability during a voyage. However, this practice posed a significant environmental risk, as it facilitated the transport of marine organisms from one ecosystem to another. To mitigate this impact, international regulations were implemented, including the D-2 standard established by the International Maritime Organization (IMO), which requires the reduction or elimination of living organisms through onboard treatment systems. In this context, the present study aimed to detect pathogens in the ballast water of ships in Colombian seaports, to verify compliance with Resolution 477 of 2012 issued by the General Maritime Directorate (DIMAR). The study included vessels that arrived at various Colombian seaports. The methodology used was to take samples directly from the ballast water tanks in order to detect indicator microorganisms defined by the D-2 standard, specifically *Vibrio cholerae, Escherichia coli* (E. coli) and intestinal enterococci, as well as the study of ship registry documentation to counterbalance the validity of the research. The results showed that Vibrio cholerae presented a concentration of <1 colony-forming unit (CFU) per 100 ml in all monitored years (2020 to 2023). In the case of E. coli, growth was evidenced in one ballast water tank in the year 2023; however, the values remained within the permissible limits established by the regulation. For its part, there was growth of intestinal enterococci exceeding the 100 CFU/100 ml limit stipulated by the D-2 standard in two sampled ships, in the years 2020 and 2023. In the same way, the vessels comply with the records updated to the date of the implementation of the treatment systems. This study reinforces the importance of having regulations that allow for comprehensive management of environmental sustainability in the oceans, and that, when regulated, monitoring is continuous.

KEYWORDS: Ballast water, ship, water filtration, microorganism, ports, maritime transport.

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Resumen

El agua de lastre fue utilizada históricamente en las embarcaciones para mantener su estabilidad durante la navegación. Sin embargo, esta práctica representó un riesgo ambiental significativo, ya que facilitó el transporte de organismos marinos de un ecosistema a otro. Para mitigar este impacto, se implementaron regulaciones internacionales, entre ellas la norma D-2, establecida por la Organización Marítima Internacional (OMI), la cual exige la reducción o eliminación de organismos vivos mediante sistemas de tratamiento a bordo de los buques. En este contexto, el presente estudio tuvo como objetivo realizar la detección de patógenos del agua de lastre en puertos marítimos colombianos, con la finalidad de verificar el cumplimiento de la Resolución 477 de 2012, emitida por la Dirección General Marítima (Dimar). El área de estudio incluyó los buques que arribaron a diversos puertos marítimos de Colombia. La metodología empleada fue la toma de muestras directamente de los tanques de agua de lastre para la detección de microorganismos indicadores definidos por la norma D-2, específicamente Vibrio cholerae, Escherichia coli (E. coli) y enterococos intestinales, así mismo el estudio de la documentación de registros de buques para contrarrestar la validez de la investigación. Los resultados mostraron que Vibrio cholerae presentó una concentración <1 unidad formadora de colonia (UFC) por 100 ml en todos los años monitoreados (2020 a 2023). En el caso de E. coli, se evidenció crecimiento en un tanque de agua de lastre en el año 2023; no obstante, los valores se mantuvieron dentro de los límites permisibles establecidos por la normativa. Por su parte enterococos intestinales mostró un crecimiento superior a 100 UFC/100 ml en dos buques muestreados durante los años 2020 y 2023, superando los valores estipulados por la norma D-2. De igual manera, los buques cumplen con los registros al día de la implementación de los sistemas de tratamiento. El estudio realizado refuerza la importancia de la existencia de normativas que permitan una gestión integral de la sostenibilidad ambiental en los océanos, y que al estar regulada el seguimiento es continuo.

Palabras clave: *agua de lastre, buque, filtración de agua, microorganismo, puerto, transporte marítimo.*

Introduction

International maritime transport is the backbone of global trade, moving around 80% of the world's traded goods (UNCTAD, 2017; 2023). However, this activity, which is essential to the global economy, faces significant environmental challenges that require an urgent transition to more sustainable operating models. Among these challenges, one of the most critical, yet least recognized, is the uncontrolled discharge of ballast water by international vessels: a routine process that, paradoxically, threatens marine biodiversity and human health on a global scale.

Ballast water, essential for the structural stability of ships, can transport and release thousands of aquatic organisms, from microorganisms to invasive species, into ecosystems lack natural defenses against them. This unintentional biological transfer has been associated with profound ecological impacts, alterations in ecosystem functionality, and the spread of pathogens (Ruiz *et al.*, 2000; Davidson, Minton, Carney, Miller & Ruiz, 2017).

In response to this emerging risk, the international community has strengthened regulations on ballast water management, particularly through the implementation of Regulation D-2 of the International Convention for the Control and Management of Ships' Ballast Water and Sediments, established by the IMO, which aims to ensure that ballast water management complies with microbiological criteria and the respective discharge limits into the sea, as shown in Table 1 (IMO, 2004).

The three target microorganisms are recognized pathogens. *Vibrio cholerae* is part of the aquatic microbiota and has more than 200 known serogroups, only two of which cause cholera; other serogroups do not cause cholera but can cause bloody diarrhea, gastroenteritis and extraintestinal infections (Bakalar, 2016). For its part, *E. coli* is a bacterium that normally inhabits the intestines of humans and warm-blooded animals, and some of its strains can cause gastrointestinal, urinary, or systemic diseases. Its presence in environmental waters is considered an indicator of fecal contamination,

and it is discharged into the environment through faeces or wastewater effluents (Jang *et al.*,2017).

Enterococci are opportunistic bacteria that can cause a large number of infections in humans and animals. Since they are excreted in faeces, they are commonly found in contaminated water and are easy to culture in the laboratory. These characteristics have favored their use as microbiological indicators of fecal contamination and as substitutes for waterborne pathogens in research studies and water quality monitoring programs around the world, especially in contexts of exposure to recreational waters (Byappanahalli *et al.*, 2012).

Furthermore, in Colombia, DIMAR, through Resolution 477 of 2012, has adopted specific verification and monitoring measures, with technical support from the Oceanographic and Hydrographic Research Center of the Caribbean (CIOH), to control the entry of invasive organisms via Colombian Caribbean ports.

In this context, the present study aimed to evaluate the presence and concentration of pathogenic microorganisms in ballast water discharged by international vessels in Colombian Caribbean ports, in order to establish compliance with current international and national standards and provide technical evidence to guide decision-making in the environmental management of maritime transport. This research thereby seeks to contribute to the protection of marine ecosystems, while also strengthening the role of Colombian ports as responsible actors in sustainable maritime trade.

Table 1. Discharge limits as set out in the IMO D-2 standard

Microorganism	Discharge limit
Vibrio cholerae	<1 UFC/ 100 ml
Escherichia coli	<250 UFC/ 100 ml
Intestinal enterococci	<100 UFC/ 100 ml

Study area

During the years 2020-2023, fifteen (15) international vessels arriving at the ports of Coveñas (Sucre), Cartagena (Bolívar), Puerto Bolívar (La Guajira), Santa Marta (Magdalena) and Barranquilla (Atlántico), whose locations are shown in Figure 1, were randomly selected for the purpose of verifying their ballast water management.

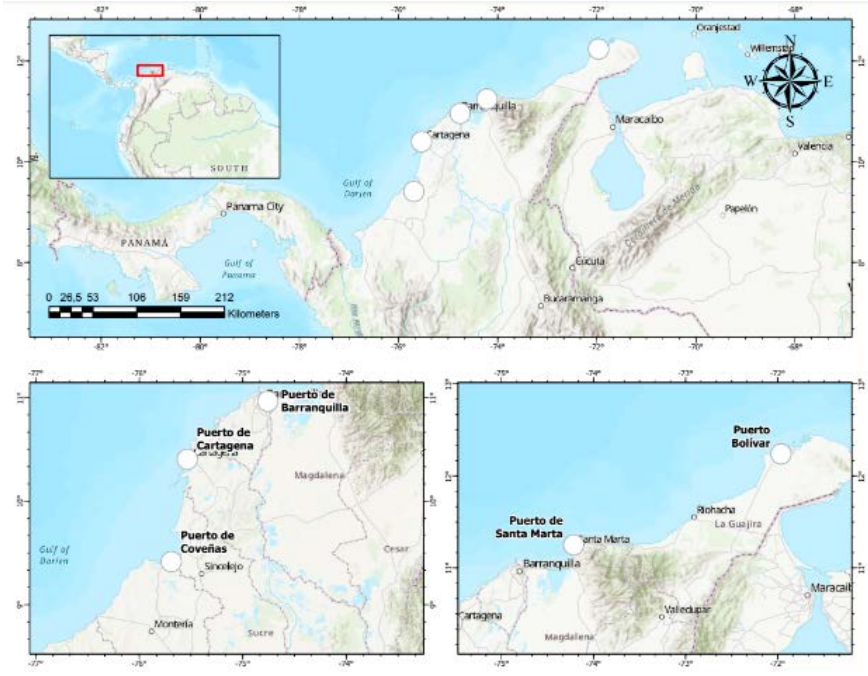

Figure 1. Location of the ports Coveñas-Sucre, Cartagena-Bolívar, Puerto Bolívar-La Guajira, Santa Marta-Magdalena and Barranquilla-Atlántico.

Methodology

Microbiological sampling

We used 500 ml Schott® glass bottles to collect microbiological samples. Prior to the start of the research, they underwent a special washing and disinfection process to ensure sterile conditions during sampling. They were then packed and labeled with Kraft paper to ensure correct identification.

Sampling was carried out by collecting ballast water from the ships' manholes using a Niskin bottle. This equipment was inserted into the ballast tank until the desired depth was reached, allowing a representative sample to be taken. Once collected, the water was transferred to the Schott® bottles, leaving an air space inside each bottle to facilitate homogeneous agitation of the sample during the subsequent analysis in the laboratory (Fig. 2).

Finally, the samples were stored in portable coolboxes with ice, ensuring a storage temperature below 10°C while they were transported to the laboratory.

Figure 2. Microbiological sampling of ballast water on board ships

Processing of microbiological samples

The samples were prepared in the field, following the protocols established by the Dimar laboratory at its Caribbean Headquarters, in order to preserve the integrity of the samples and ensure reliable microbiological results.

The analytical techniques used for each parameter are described in Table 2. Microorganism detection methods have varied over the years, in accordance with the laboratory procedures in force at the time (Dimar). However, all of them are considered valid and meet the technical standards and capabilities that were applicable at the time.Microbiological analyses focused on the detection and quantification of *Vibrio cholerae*, *E. coli*, and intestinal enterococci as indicators of microbial contamination in ballast water.

Table 2. Analytical techniques used to detect *Vibrio cholerae, E. coli* and intestinal enterococci

Year of sampling	Parameter	Technique	Reference standard	General description
2020 - 2021	*Vibrio cholerae*	Membrane filtration	Modified SM 9260H (APHA/AWWA/ WEF,2017).	**Enrichment:** 1 % Alkaline Peptone Water **Agar:** TCBS, BHI, CromoAgar **Incubation temp:** 35±2°C **Incubation time:** 18 to 24 hours **Biochemical tests:** String and oxidase
	Escherichia coli	Membrane filtration	SM 9222D (APHA et al., 2017).	**Selective agar isolation:** m-FC **Incubation temp:** 44.5 ± 0.2°C **Incubation time:** 24±2 hours **Confirmation tests in:** EC-MUG broth, Lauryl Tryptose broth, BRILA broth, Indol and Citrate
	Intestinal enterococci	Membrane filtration	SM 9230C (APHA et al., 2017).	**Selective agar isolation:** m-Enterococcus, BHI **Incubation temp:** 35±0.5°C Incubation time: 48± 3 hours **Confirmation tests in:** BHI broth, Bile esculin agar, BHI broth, BHI broth with 6.5 % NaCl
2023	*Vibrio cholerae*	Membrane filtration	Modified SM 9260H (APHA, et al., 2017).	**Enrichment:** 1 % Alkaline Peptone Water **Agar:** TCBS, BHI, CromoAgar **Incubation temp:** 35±2°C **Incubation time:** 18 to 24 hours **Biochemical tests:** String and oxidase
	Escherichia coli	Membrane filtration	Modified ISO 9308-1:2014/A1:2017 (International Organization for Standardization, 2017).	**Selective agar isolation:** CCA **Incubation temp:** 36 ± 2°C **Incubation time:** 21-24 hours **Confirmation tests in:** Indol
	Intestinal enterococci	Membrane filtration	UNE-EN ISO 7899-2:2000 (Asociación Española de Normalización, 2000).	**Selective agar isolation:** Slanetz and Bartley agar (m-Enterococcus agar) **Incubation temp:** 36±2°C Incubation time: 44± 4 hours **Confirmation tests in:** Bile esculin agar

Documentary evidence support

To support the findings of this research, various documentary sources were collected and analyzed, such as ship records, documentation traceability, and current regulations related to the subject of study. These documents were selected for their relevance and reliability, with their authenticity verified through the review of official sources.

The documentary evidence was mainly used to contrast the information obtained through interviews and observations, allowing for data triangulation and strengthening the validity of the results. All documentation was safeguarded in both digital and physical formats, in accordance with the confidentiality protocols established for the research.

Results

During the period 2020-2023, there was no growth of *Vibrio cholerae* in any of the tanks sampled on the fifteen (15) ships selected. *E. coli* growth was only detected in one of the samples: tank WBT4S of the UBC Savannah, sampled in the port of Barranquilla in 2023, with a result of 3 CFU/100 ml. Nevertheless, this concentration is below the limit permitted by Dimar Resolution 477 of 2012.

In contrast, the presence of intestinal enterococci was more frequent, being detected in seven (7) of the fifteen (15) ships analyzed during the period. In two of these cases, the concentration exceeded the maximum values established in the regulations: the ship Jackeline C., in the port of Cartagena (2020), with 152 CFU/100 ml; and UBC Savannah, in the port of Barranquilla (2023), with 158 CFU/100 ml, as shown in Table 3.

Table 3. Results of the microorganisms found in the ballast water from the ships sampled during the period 2020-2023.

Year	Area	Ship	Tank	*Vibrio cholerae* (CFU/100ml)	E. coli (CFU/100ml)	Intestinal enterococci (CFU/100ml)
2020	Coveñas	Eagle Kinarut	3BS	<1	<1	2
			6BS	<1	<1	22
			6P	<1	<1	23
	Cartagena	Velos Aquarius	3 PORT WGT	<1	<1	<1
			4 PORT WGT	<1	<1	9
			3 STBD WGT	<1	<1	3
			4 STBD WGT	<1	<1	5
		Velebit	WBT 4P	<1	<1	<1
			WBT 2P	<1	<1	<1
			WBT 2S	<1	<1	<1
		Jackeline C.	3SWBT	<1	<1	152
			5SWBT	<1	<1	97
			5BWBT	<1	<1	53
		Silver Manoora	5WBTS	<1	<1	<1
			5WBTP	<1	<1	7
		Aurora N	T5	<1	<1	<1
			T4P	<1	<1	<1
			T3S	<1	<1	<1

Year	Area	Ship	Tank	*Vibrio cholerae* (CFU/100ml)	E. coli (CFU/100ml)	Intestinal enterococci (CFU/100ml)
2021	Santa Marta	Elizabeth II	5 TST	<1	<2	<1
			6 TST	<1	<2	<1
			4 TST	<1	<2	<1
		Tiger South	WBT 5	<1	<2	<1
			WBT 4	<1	<2	<1
		BW Japan	1TSWB	<1	<2	<1
			3TSWB	<1	<2	<1
	Puerto Bolívar	Algoma Victory	ST1P	<1	<2	1
			ST1S	<1	<2	<1
		Green Universe	ST 3T	<1	<2	3
			ST 1T P	<1	<2	8
			ST 1T S	<1	<2	<1
		UBC Santa Marta	4WB P	<1	<2	<1
			4WB S	<1	<2	<1
2023	Cartagena	CMA CGM BERLIOZ	NO5 SWBT(P)	<1	<1	<1
			NO5 SWBT(S)	<1	<1	<1
		Polar Brasil	4BWTP	<1	<1	<1
			4BWBTS	<1	<1	<1
	Barranquilla	UBC Savannah	WBT4S	<1	3	15
			WBT4P	<1	<1	158

Note. *The results highlighted in gray show that microorganisms were present, while those highlighted in red exceeded the permitted values*

During the analysis of the results, the documentary evidence collected—such as technical maintenance records, the traceability of ship documentation, and current regulations—served as complementary evidence to contrast the information obtained through interviews and observations.

These documents made it possible to verify the correspondence between the practices reported by the personnel and the procedures officially established, highlighting both consistencies and gaps in the application of ballast water treatment systems. The documentary review also provided regulatory and technical context that strengthened the interpretation of the findings and allowed for a better understanding of the variations observed among different vessels.

These findings reflect compliance with the parameters established by international regulation D-2 for *Vibrio cholerae* and *E. coli*, but warn of recurrent detection of non-compliant levels of intestinal enterococci. This may indicate specific deficiencies in treatment systems or in on-board maintenance and monitoring protocols. (IMO, 2004).

Discussion

According to the results obtained from monitoring the ballast water tanks of ships that arrived at Colombian ports between 2020 and 2023, it was found that the concentrations of the microbiological indicators *Vibrio cholerae* and *Escherichia coli* complied with the permissible limits established in Regulation D-2 of the International Convention for the Control and Management of Ships' Ballast Water and Sediments (IMO, 2004). However, the presence of intestinal enterococci was detected on the vessels Jackeline C. and UBC Savannah, which were sampled in the ports

of Cartagena (2020) and Barranquilla (2023), respectively.

This situation could be related to variability in the efficiency of ballast water treatment systems, which can perform poorly or well depending on their design, maintenance and operating conditions (Bakalar, 2016).

According to the documentation submitted by the ships' crews, most of the treatment systems use combined mechanical filtration and ultraviolet (UV) radiation technologies; this is an effective method, albeit with limitations against certain resistant organisms, such as some types of phytoplankton and zooplankton. Bacteria, on the other hand, tend to be more sensitive to UV radiation (Nanayakkara *et al.*,2011). Various studies have shown that the combination of mechanical separation and UV radiation increases the rate of microorganism inactivation (Hess-Erga *et al.*, 2019; Romero-Martínez *et al.*, 2014).

In contrast, the UBC Savannah uses a filtration system combined with electrochlorination, a technology considered highly effective for ballast water disinfection, particularly in scenarios where there is a high microbiological risk. This system generates chlorine in situ by electrolysis of a saline solution, which enables the inactivation of bacteria, viruses and other pathogens (Hess-Erga *et al.*, 2019). While various studies have demonstrated its effectiveness against the planktonic organisms covered by the D-2 standard, its main disadvantage is its high operating cost (First *et al.*,, 2016; Tsolaki & Diamadopoulos, 2010).

The detection of bacterial growth in these ships could be related to operational failures in the treatment systems, such as poor maintenance, inadequate salt or chlorine concentrations, or the presence of biofilms in the pipes, which compromises the effectiveness of the process (Stehouwer *et al.*,2015).

Intestinal enterococci were the most prevalent of the bacteria studied, as they are microorganisms that tolerate adverse conditions such as pH changes, high salt concentrations and the presence of disinfectants; they also survive better in contaminated water and soil compared to other species of the same genus (Byappanahalli *et al.*, 2012).

The detection of pathogens in the ballast water of international shipping vessels is essential due to its importance in sanitation, as it contributes to the prevention of infectious diseases. A contaminated vessel can become a source of outbreaks on board and there is a risk of these spreading at the port of destination. From an environmental perspective, this control prevents the introduction of invasive species and microorganisms that alter local marine ecosystems and displace native species, thereby reducing the negative impacts on fauna and flora caused by non-native microorganisms (Sellera *et al.*,2024).

Finally, this study constitutes a first attempt to demonstrate the current state of compliance with Regulation D-2 by ships entering Colombian Caribbean ports (IMO, 2004).

As a projection for future research, this study provides a methodological basis for establishing correlations between process traceability and control systems, considering whether they may be applicable as performance indicators in microbiological testing.

Conclusions

The results obtained during this research confirmed the initial hypothesis: adequate monitoring of the ballast water used by international shipping is essential to mitigate the introduction of non-native species and protect marine ecosystems. The analysis of microbiological indicators revealed the potential presence of microorganisms that could alter the natural balance of aquatic ecosystems, confirming the need for rigorous monitoringl of ballast water. The study also found that the treatment systems used by ships not only comply with current regulatory requirements, but also optimize the operational efficiency of these vessels by reducing the pollutant load before discharge.

This research will enable future lines of work to be carried out, such as the development of more efficient technologies for the treatment of ballast water, comparative studies between different treatment systems, and the assessment of long-term ecological impacts in regions exposed to international maritime traffic. The findings presented here reinforce the importance of comprehensive and proactive management

in international maritime transport, aimed at preserving environmental health and the sustainability of the oceans.

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Author contributions

Conceptualization: N.N.C.L.; K.L.S.; Methodology:N.N.C.L.; Formal analysis: N.N.C.L.; K.L.S.; Writing – original draft: N.N.C.L.; Writing – review & editing:K.L.S.; Supervision: K.L.S.

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