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A Hybrid Modeling Framework for Predicting Spatiotemporal Dynamics of Antimicrobial Resistance in Coastal Waters

Xuneng Tong,* Zhixin Xiang, Shin Giek Goh, Luhua You, Sanjeeb Mohapatra, Glendon Ong, Wei Ching Khor, Kyaw Thu Aung, and Karina Yew-Hoong Gin*



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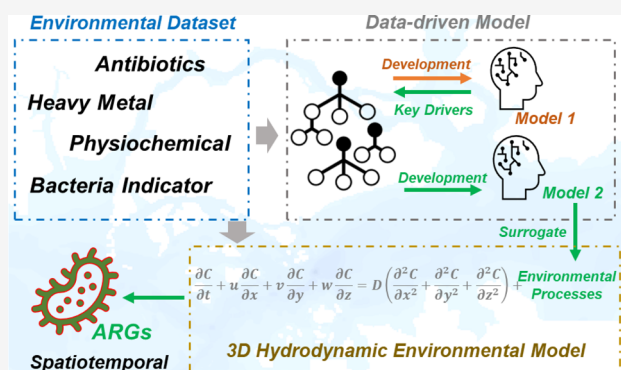
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ABSTRACT: Antimicrobial resistance (AMR) in aquatic environments poses a critical threat to both environmental and human health. This study presents a novel hybrid modeling framework that integrates a process-based hydrodynamic-environmental model with a data-driven approach to predict the spatiotemporal dynamics of AMR in coastal waters. Macrolide-related antimicrobial resistance genes (ARGs_Macro) were selected as representative markers. The model results were validated using data from a monthly sampling campaign conducted across Singapore's coastal waters, yielding a mean coefficient of determination (R^2) of 0.693, a Nash-Sutcliffe efficiency (NSE) of 0.589, and a root-mean-square deviation (RMSE) of 0.0257 GC/16S rRNA across 12 sampling points. Lincomycin, pH, dissolved oxygen, zinc and temperature were identified as significant influencers of ARGs_Macro. Although Lincomycin is not classified as a macrolide, it ranks as the most important driver of ARGs_Macro due to its shared resistance mechanisms with macrolides, potentially facilitating cross-resistance. The spatiotemporal model results revealed that coastal areas, particularly in the northern part of Singapore, are vulnerable to significant ARG accumulation, with monsoon seasons amplifying the spread of AMR due to hydrodynamic conditions. This study highlights the development of a robust modeling framework that provides valuable insights into the environmental drivers of AMR in coastal waters, offering a foundation for regulatory strategies and future research aimed at mitigating the risks of antimicrobial resistance in aquatic environments.

KEYWORDS: antimicrobial resistance, antibiotic resistance genes, hybrid modeling, hydrodynamic-environmental model, data-driven model



1. INTRODUCTION

The global proliferation of antimicrobial resistance (AMR) has been widely recognized as one of the most significant global health and development threats.¹ It is estimated that bacterial AMR was associated with approximately 4.95 million deaths in 2019, including around 1.27 million deaths directly attributable to bacterial AMR.² The aquatic environment can act as a reservoir for antimicrobial-resistant bacteria (ARB) and antimicrobial resistance genes (ARGs), fueled by the influx of AMR-related pollutants, such as antibiotic residues from agriculture, aquaculture and wastewater discharge.³ These pollutants facilitate the development of AMR through interactions with the surrounding environment, either synergistically or antagonistically.⁴ Aquatic environments also serve as transmission pathways, where ARGs and ARB can reach humans and animals through direct contact, contaminated water or food consumption.⁵ Therefore, it is essential to deepen our understanding of AMR in aquatic environments to effectively mitigate its spread and protect both environmental and public health.

Coastal waters, due to their proximity to both urbanized and agricultural areas, are particularly vulnerable to the accumulation of AMR pollutants.⁶ In addition, intensive aquaculture activities may increase the burden of AMR in coastal areas, given that antibiotics may be used for maintenance of fish health.⁷ The coselection pressure exerted by heavy metals and other pollutants often found in coastal waters can enhance the survival and spread of AMR,⁸ while diverse microbial communities in these regions also act as hotspots for horizontal gene transfer, further driving the dissemination of AMR.⁹ At the same time, the complex hydrodynamics of coastal zones, characterized by tides, currents and mixing patterns, can influence the distribution and persistence of

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AMR-related contaminants.¹⁰ The combination of multiple anthropogenic sources and influencing factors affecting AMR in coastal waters thus presents significant challenges in understanding its dynamics.

To effectively address AMR-related issues, it is essential to first understand their sources, fate, transport and eventual destinations in aquatic environments.¹¹ While occurrence studies of AMR in various ecosystems have provided early warning to scientists and policymakers,¹² the analysis of AMR pollutants remains highly dependent on specialized expertise and advanced equipment.¹³ For example, current ARG testing often requires molecular techniques like quantitative polymerase chain reaction¹² and next-generation sequencing,¹⁴ which are costly and resource-intensive, thereby limiting effective data collection.¹⁵ Large-scale water body sampling is particularly time-consuming and expensive.¹⁶ Additionally, maintaining in situ sampling for high-frequency and spatially resolved data is challenging, further complicating efforts to effectively monitor AMR.¹⁷ As a result, field monitoring campaigns often fail to provide a comprehensive picture of AMR distribution across aquatic environments. Hence, developing novel approaches to overcome the burdens of expensive, labor-intensive laboratory analyses and extensive sampling campaigns is vital for improving the management of AMR pollution in aquatic environments.

The hydrodynamic-environmental models (HEMs) have emerged as a valuable tool for understanding the behavior of pollutants in aquatic systems across both temporal and spatial scales.¹⁸ By integrating various hydrodynamic and biogeochemical processes, HEMs have been widely applied to simulate and predict the transport and fate of a diverse range of contaminants, including heavy metals,¹⁹ organic chemicals,²⁰ microbial contaminants²¹ and across different water systems. However, the application of HEMs in predicting AMR in aquatic environments is still in its infancy, as the mechanisms conferring AMR in these systems are not yet fully understood.²² This knowledge gap makes it challenging to develop accurate mathematical models that can effectively describe the complex processes involved in the transport, persistence and dissemination of AMR.²³ Unlike process-based HEMs, data-driven models (DDMs), such as machine learning, do not require a detailed understanding of the underlying processes; they rely solely on input and output data.²⁴ Recently, DDMs have shown great promise in predicting AMR levels in aquatic environments by analyzing potential nonlinear relationships between environmental parameters (e.g., heavy metals, dissolved oxygen, antibiotics) and AMR. Jang et al. predicted the occurrence and distribution of ARGs at a recreational beach in South Korea with machine learning models.²⁵ Similar machine learning approaches have also been applied across various environments to predict AMR dynamics.²⁶ However, due to the empirical nature of DDMs, they heavily rely on historical data sets,²⁷ limiting their ability to predict AMR levels only at specific points where sampling data is available. This dependence on existing data constrains the models' generalizability and their effectiveness in predicting AMR distribution across unsampled areas. Additionally, DDMs are unable to predict the spread and movement of contaminants through water, a key aspect that can be effectively modeled using HEMs, which simulate the transport and fate of pollutants over time and space.²⁸ Hence, a hybrid modeling approach that combines the strengths of both HEMs and DDMs offers an optimized solution for predicting the

dynamics of AMR in aquatic environments, especially in large-scale water bodies such as coastal waters and lakes.

Macrolide antibiotics are categorized by the World Health Organization as "Highest Priority, Critically Important Antimicrobials" due to their vital role in treating severe bacterial infections.²⁹ Macrolides are especially important in cases where other treatment options are limited or ineffective, making them essential in both human and veterinary medicine.³⁰ The rising prevalence of macrolide-resistant bacteria, driven by the spread of macrolide-related ARGs, poses a significant public health risk by compromising the effectiveness of these critical antibiotics.³¹

The objective of this study was to establish a modeling framework by integrating process-based HEMs with DDMs to predict the transport and fate of AMR over time and space in aquatic environments impacted by anthropogenic inputs. The macrolide-related ARGs, i.e., *mphA* and *mefA*, were selected as representative ARGs. This framework was applied to Singapore's coastal waters and calibrated and validated using a comprehensive environmental data set from a one-year sampling campaign. The model results identified spatiotemporal hotspots of AMR and provided a detailed understanding of various factors, including meteorological, hydrodynamic and biogeochemical processes, that influence the development and propagation of AMR in coastal ecosystems. The new insights revealed by the integrated modeling framework can serve as a valuable early warning toolbox for identifying spatiotemporal AMR hotspots.

2. MATERIALS AND METHODS

2.1. Study Area and Environmental Data Set.

Singapore is an equatorial island nation with shared coastal waters with Malaysia and Indonesia. The climate of Singapore is mainly influenced by two primary monsoon seasons, interspersed with two transitional intermonsoon periods. The Northeast (NE) Monsoon prevails from December to March, succeeded by the first intermonsoon period spanning April to May. The Southwest (SW) Monsoon extends from June to September, followed by the second intermonsoon period in October and November. During the NE Monsoon, winds predominantly blow from the northeast, while in the SW Monsoon, the prevailing winds shift to the southeast.

Based on the occurrence data (Table S1 and Figure S1), we found that macrolides (erythromycin-H₂O (ERY-H₂O), azithromycin (AZT) and clarithromycin (CLAR)) and one lincosamide antibiotic (Lincomycin (LIN)) were the most frequently detected antibiotics in the study area. These antibiotics exhibited a wide concentration range, with ERY-H₂O reaching up to 5948 ng/L and AZT up to 25.69 ng/L, indicating their widespread presence in the aquatic environment. The detection frequencies of macrolides were also notably high, particularly for CLAR (98%) and AZT (75%), further confirming their persistence and potential ecological impact. Given the significant occurrence and persistence of macrolides and lincosamides, their corresponding antibiotic resistance genes (ARG_Macro) are likely to be present and actively selected for in microbial communities. These findings justify ARG_Macro as a suitable modeling target to assess its environmental fate, potential risks, and implications for AMR management.

The environmental data set includes 18 categories of variables to comprehensively capture factors influencing ARGs_Macro in aquatic environments (Table 1). The six

Table 1. Variable Type, Abbreviation, Full Name Description, and Units of Environmental Variables

variable type	variable abbreviation	variable description	unit
physicochemical variables	temp	water temperature	°C
	pH	pH value	
	Sal	Salinity	ppt
	DO	dissolved oxygen	mg/L
	TDS	total dissolved solids	mg/L
microbial indicators	TUR	Turbidity	NTU
	<i>E. coli</i>	<i>Escherichia coli</i>	MPN/100 mL
macrolide antibiotics	<i>Enter</i>	<i>Enterococcus</i>	MPN/100 mL
	ERY-H ₂ O	erythromycin-H ₂ O	ng/L
	AZT	azithromycin	ng/L
	CLAR	clarithromycin	ng/L
heavy metals	LIN	lincomycin	ng/L
	Cd	cadmium	ppb
	Cu	copper	ppb
	Pb	lead	ppb
antibiotic resistance gens	Zn	zinc	ppb
	<i>mphA</i>	ARGs corresponding to macrolides	GC/16S rRNA
	<i>mefA</i>	ARGs corresponding to macrolides	GC/16S rRNA

physicochemical variables (e.g., temperature, pH) affect bacterial growth and gene transfer,³² while two microbial indicators provide insight into potential carriers of ARGs.³³ The three macrolide antibiotics are directly linked to ARGs_Macro, and lincomycin, a lincosamide, is included because it shares similar mechanisms of resistance with macrolides and may lead to cross-resistance.³⁴ Additionally, four heavy metals are included as they may coselect for ARGs, aiding in their persistence and spread.³⁵ This diverse data set ensures a robust prediction of ARGs_Macro and was obtained from a sampling campaign conducted at 12 sampling sites across Singapore coastal waters (Figure 1) from January 2022 to January 2023 with a total of 144 water samples. Six physicochemical parameters (i.e., temperature (Temp), pH, salinity (Sal), dissolved oxygen (DO), total dissolved solids (TDS) and turbidity (Tur)) were measured in situ using an

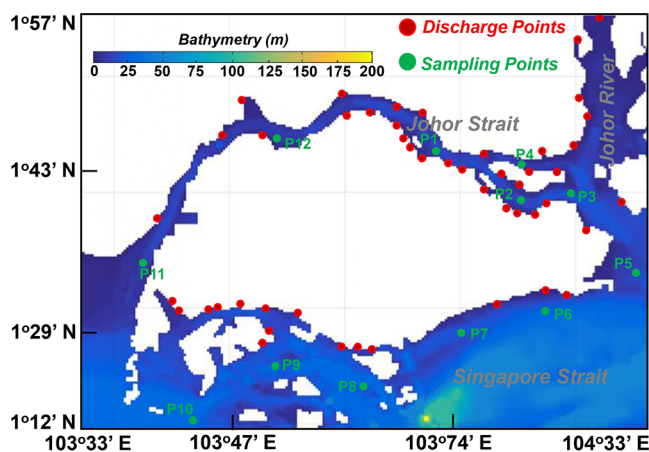


Figure 1. An overview of the study area showing riverine discharge points and sampling points.

EXO2 sensor (YSI, Inc.). The water samples for the other environmental variables were collected using plastic bottles from the surface layer at a depth of 0–0.5 m as follows: 1 L for microbial indicators, 1 L for antibiotics, 0.5 L for heavy metals and 40 L for ARGs. All the water samples were kept in the dark at 4 °C cold room until analysis. The two microbial indicators, *Escherichia coli* and *Enterococcus*, were measured with Colilert (IDEXX Laboratories, Inc., Westbrook, Maine). Furthermore, three macrolide antibiotics (ERY-H₂O, AZT and CLAR) and one lincosamide antibiotic (Lincomycin (LIN)) in the dissolved phase were analyzed by a combination of solid phase extraction and ultrahigh-performance liquid chromatography-tandem mass spectrometry (SPE-UPLC/MS/MS). Nguyen et al. (2019) comprehensively reviewed the relationship between environmental heavy metals and antibiotic resistance in aquatic environment, identifying Zinc (Zn), Cadmium (Cd), Copper (Cu), and Lead (Pb), particularly Zn and Cd, as the most frequently linked to antibiotic resistance.³⁶ Given this evidence and the availability of relevant data, we prioritized four heavy metals including Zn, Cd, Cu, and Pb for analysis using inductively coupled plasma mass spectrometry (ICP-MS) (Agilent 7700 series, USA). The ARGs corresponding to macrolides (*mphA* and *mefA*) were analyzed using a Qubit spectrophotometer and analyzed with high throughput qPCR (HT-qPCR). The detailed sampling and analytical methods can be found in the Supporting Information and our previous study.⁶

2.2. Modeling Framework. A hybrid modeling framework coupling a process-based three-dimensional hydrodynamic-environmental model (HEM) and a data-driven model (DDM) was proposed to predict the spatiotemporal presence and abundance of macrolide-associated ARGs in Singapore coastal waters. This comprehensive framework follows 13 sequential steps (Figure 2), combining mechanistic and statistical approaches for robust AMR prediction.

2.2.1. Data-Driven Model. The Random Forest (RF) algorithm, a well-regarded ensemble machine learning technique was applied to explore the relationships between environmental variables and ARGs. The Random Forest algorithm is robust and excels in predictive tasks, making it an apt choice for our research objective. Model development began with comprehensive data preparation (Step 1), where 16 input variables including six physicochemical parameters, two microbial indicators, four antibiotics (three macrolides and one lincosamide), and four heavy metals were compiled to predict the target output variable (ARGs_Macro), defined as the sum of *mphA* and *mefA* gene abundances. The data set was strategically partitioned into training (70%), validation (15%), and testing (15%) subsets to ensure proper model evaluation. The model was trained on training data, allowing it to learn the intricate relationships between the physicochemical parameters, microbial indicator bacteria, antibiotics, heavy metals and ARG concentrations. We also performed hyperparameter tuning guided by the validation set, a critical step in optimizing the model's performance. Tuning parameters such as the number of trees, maximum depth, and minimum samples per leaf were conducted to ensure that the Random Forest model was finely tuned for its predictive task. Then, the testing set was held out for final performance assessment to ensure unbiased evaluation. The modeling proceeded through two sequential stages: Model 1 initially incorporated all 16 environmental variables (Step 2), with its performance quantitatively assessed using *R*-squared (*R*²), Nash-Sutcliffe efficiency (NSE) and root

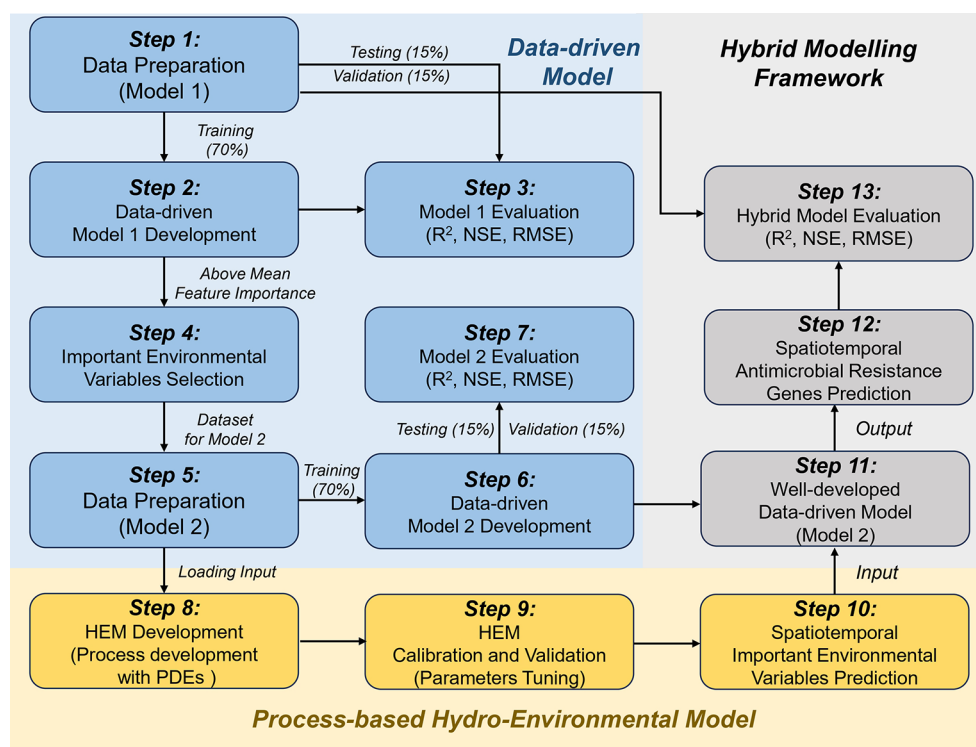


Figure 2. Schematic flowchart of the hybrid modeling framework.

mean squared error (RMSE) metrics (Step 3). The relative importance of these environmental variables was assessed in the interim using the permutation method, which calculates the average change in mean square error of the model fit upon random shuffling of a variable ten times. Environmental variables with feature importance scores above the mean in Model 1 were retained as training inputs for Model 2 (Step 4). Then, Model 2 was trained, tested and validated using the same development protocols as Model 1 (Steps 5–7). Detailed description of the model performance evaluation and data driven model can be found in [Supporting Information](#).

2.2.2. Process-Based Hydrodynamic Environmental Model (HEM). A 3D HEM was developed based on the Delft 3D software suite (<http://oss.deltares.nl/web/delft3d>) to predict the dynamics of four important environmental variables (Temp, DO, Zn, and LIN) identified in Step 4 over time and space. The data set formed in Step 5 provides the necessary input data for Step 8 of the framework. In addition to the above parameters simulated through the hydrodynamic-environmental model, the other key driver, pH, was estimated using the mean value from a linear interpolation of the time function. The overall model consisted of a hydrodynamic and a water quality module. The hydrodynamic module simulated coastal hydrodynamic conditions driven by the tidal and meteorological forcing through Delft 3D-Flow, as provided in the [Supporting Information](#). The computational results from hydrodynamic model were then delivered to the water quality model which was used to calculate the transport of the targeted environmental variables. The water quality module simulated the fundamental environmental processes for the modeled substances that described their dynamics and behavior through Delft 3D-WAQ. All the modeled substances were governed by the advection-diffusion-reaction processes. Following comprehensive calibration and validation with R^2 , NSE, and RMSE evaluation (Step 9), the HEM provided high-resolution

environmental predictions, the HEM provided high-resolution environmental predictions. The detailed description and information on boundary conditions, governing equations and model parameters are found in the [Supporting Information](#).

2.2.3. Coupling Data-Driven Model with Process-Based Model. The final framework unified both modeling approaches through sequential coupling: validated HEM outputs (Step 10) served as dynamic inputs for the optimized DDM (Model 2, Step 11), generating spatiotemporal ARGs predictions (Step 12). This hybrid integration was evaluated against field data collected from January 2022 to January 2023, with final performance quantified using the standard evaluation metrics (Step 13). The combined approach leverages HEM's physical realism and DDM's pattern recognition capabilities for comprehensive AMR forecasting in coastal ecosystems.

3. RESULTS AND DISCUSSION

3.1. Antibiotic Resistance Gene Machine Learning Predictor. In this study, two RF models were developed to elucidate the factors influencing ARG levels in coastal waters. Model 1, dedicated to forecasting ARG_Macro levels, demonstrated reasonable predictive performance across a spectrum of evaluation metrics ([Figure 3a](#)). Specifically, the R^2 values for the training, testing and validation data sets were 0.937, 0.567 and 0.468, respectively. The precision of Model 1 was further evaluated using RMSE, a crucial metric for assessing predictive accuracy. The RMSE values for the training, testing and validation data sets were 0.017, 0.037 and 0.049, respectively, indicating that Model 1's predictions closely approximated actual values, further validating its predictive efficiency. NSE also offered an insightful perspective on Model 1's performance. The NSE values, with commendable scores of 0.892, 0.538, and 0.432 for the training, testing and validation data sets, respectively, affirmed the model's

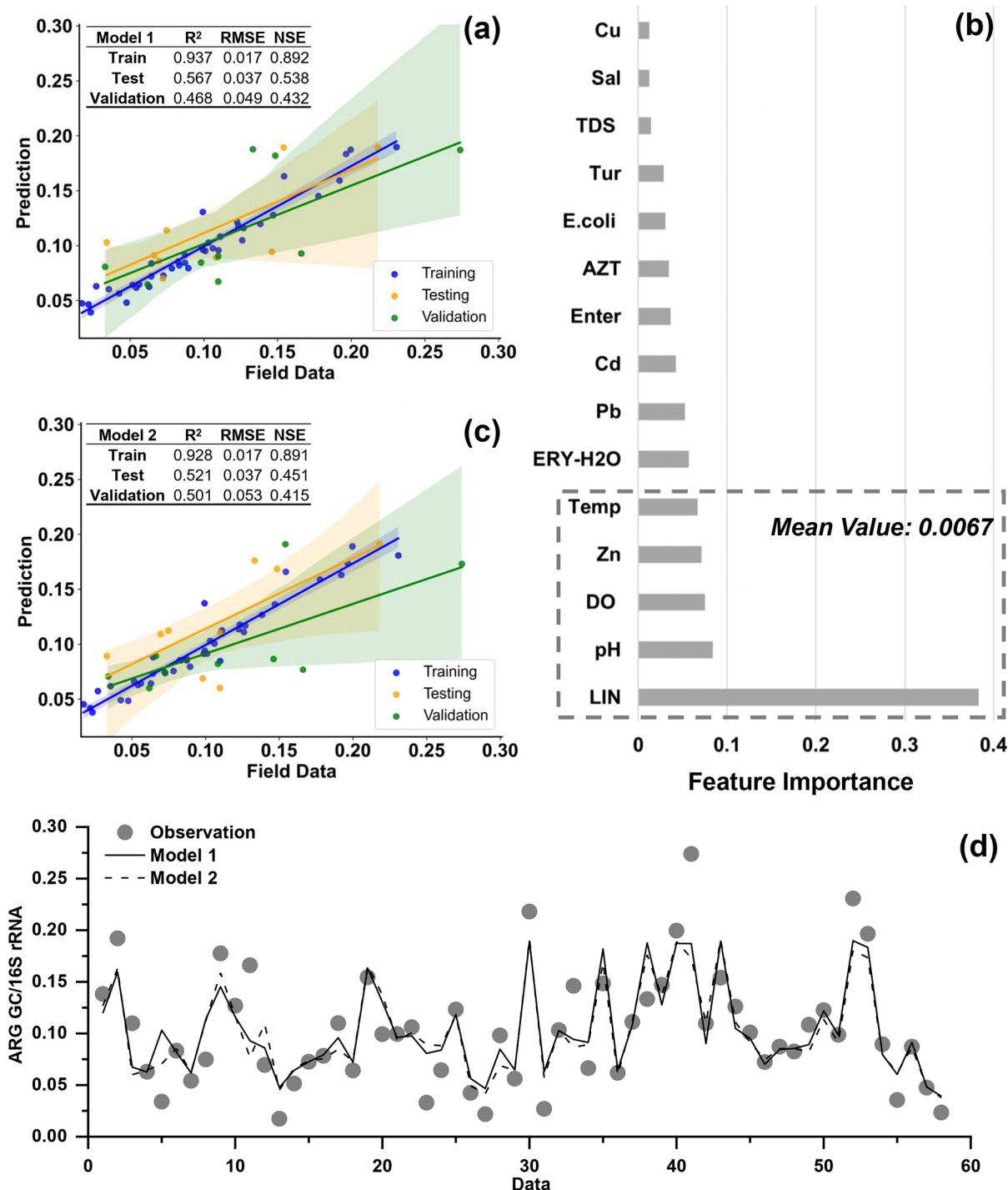


Figure 3. Results of antibiotic resistance genes machine learning predictor. (a) Prediction performance of Model 1 in emulating ARGs; (b) feature importance of Model 1; (c) prediction performance of Model 2 in emulating ARGs; and (d) comparison between Models 1 and 2.

ability to outperform a simplistic mean model, thus providing a good predictor for ARGs_Macro. These findings underscore the comprehensive and robust evaluation of Model 1, emphasizing its effectiveness in elucidating the factors influencing ARG levels in coastal waters impacted by anthropogenic inputs.

The elucidation of key drivers further enriched Model 1's predictive power. The analysis of feature importance in Model 1 revealed that LIN, pH, DO, Zn and Temp were the key variables significantly impacting ARG_Macro (Figure 3b), with feature importance values of 0.3830, 0.0836, 0.0753,

0.0711 and 0.0668, respectively, compared to the mean value of 0.0667. Despite being a lincosamide rather than a macrolide, LIN showed the highest feature importance. LIN shares functional similarities with macrolides in its mechanism of action,³⁷ which involves inhibiting bacterial protein synthesis by binding to the 50S ribosomal subunit.³⁸ This similarity can lead to cross-resistance, where the resistance mechanisms (such as those conferred by the genes *mphA* and *mefA*) are effective against both lincosamides and macrolides. The influence of pH, DO, Temp and Zn as key factors further highlights the complex interactions between environmental

Table 2. Evaluation Metrics for the Modeled Targets^a

	metrics	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	P11	P12
Temp	R ²	0.693	0.792	0.825	0.191	0.7339	0.744	0.741	0.78	0.832	0.885	0.756	0.576
	NSE	0.514	0.659	0.754	0.428	0.693	0.714	0.731	0.751	0.816	0.677	0.727	0.444
	RMSE	0.796	0.57	0.495	1.239	0.487	0.441	0.379	0.417	0.315	0.481	0.513	0.689
DO	R ²	0.959	0.919	0.639	0.94	0.723	0.143	0.209	0.963	0.852	0.897	0.775	0.469
	NSE	0.684	0.766	0.576	0.773	0.555	0.234	0.407	0.759	0.722	0.728	0.661	0.261
	RMSE	1.448	1.15	0.675	1.107	0.3	0.48	0.195	0.327	0.364	0.333	0.909	1.393
Zn	R ²	0.518	0.86	0.568	0.638	0.257	0.802	0.981	0.507	0.997	0.861	0.986	0.868
	NSE	0.417	0.742	0.607	0.495	0.439	0.745	0.831	0.235	0.875	0.859	0.865	0.713
	RMSE	0.308	0.79	0.206	0.136	0.375	0.105	0.341	0.196	0.57	0.051	0.305	0.416
LIN	R ²	0.9	0.869	0.902	0.777	0.825	0.862	0.9564	0.998	0.467	0.567	0.553	0.456
	NSE	0.875	0.764	0.823	0.75	0.794	0.774	0.546	0.462	0.136	0.712	0.483	0.219
	RMSE	0.787	0.767	0.367	0.69	0.226	0.305	0.756	1.82	1.387	0.016	0.461	0.527
ARG_Macro	R ²	0.6741	0.95	0.87	0.768	0.477	0.675	0.723	0.438	0.796	0.754	0.599	0.586
	NSE	0.48	0.801	0.766	0.678	0.421	0.322	0.699	0.437	0.706	0.69	0.533	0.535
	RMSE	0.0573	0.03	0.023	0.03	0.017	0.021	0.026	0.0159	0.016	0.016	0.036	0.02

^aThe units for Temp, DO, Zn, LIN and ARG_Macro is °C, mg/L, ppb, ng/L, and GC/16S rRNA, respectively.

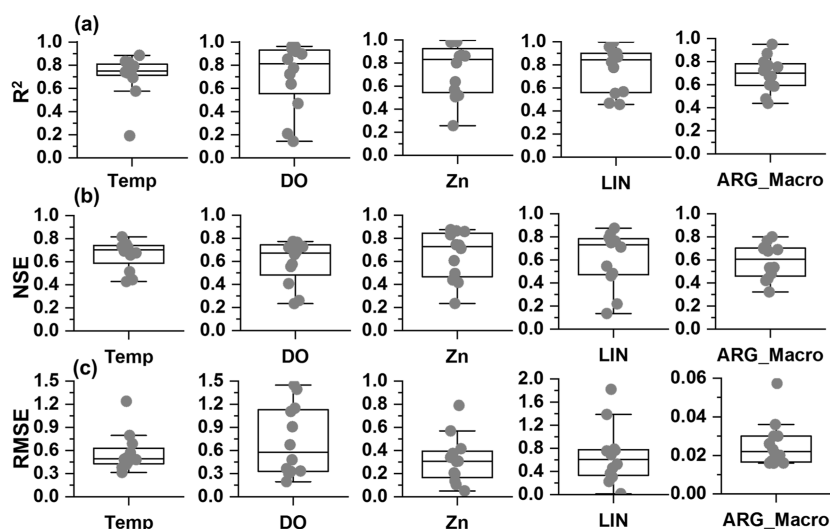


Figure 4. Evaluation metrics of the hybrid model are (a) R², (b) NSE, and (c) RMSE. The units for Temp, DO, Zn, LIN and ARG_Macro is °C, mg/L, ppb, ng/L, and GC/16S rRNA, respectively.

conditions and the mechanisms underlying antibiotic resistance. The results indicated that *E. coli* and *Enterococcus* may not be the primary carriers of ARGs_Macro in this study, as their feature importance values are below the mean value (Figure 3b). Instead, the significant role of pH and other environmental factors suggests that a broader range of microbial species or processes may be more influential in the dissemination and maintenance of these resistance genes. pH can impact the stability and activity of antibiotics and influence bacterial cell membrane integrity, thereby affecting the survival of resistant strains.³⁹ Temperature modulates microbial metabolic rates and the efficiency of horizontal gene transfer,⁴⁰ both critical in the propagation of ARGs.⁴¹ Zn, like other heavy metals, can coselect for ARGs due to the common collocation of metal resistance genes and ARGs on mobile genetic elements, facilitating their joint dissemination in the environment.⁴²

Building on the insights from Model 1, Model 2 maintained strong predictive performance in unraveling ARG dynamics by leveraging on the key drivers identified earlier (Figure 3c). The difference in prediction accuracy between the two models is minimal (Figure 3d). Model 2 exhibited reasonable predictive

performance across different phases of evaluation. During the training phase, the model performed exceptionally well with an R² of 0.928, an RMSE of 0.017, and an NSE of 0.891, indicating its strong capability to capture the underlying dynamics of the ARG system. Although the model's performance in the testing and validation phase was less robust, with an R² of 0.501–0.521, an RMSE of 0.037–0.053, and an NSE of 0.415–0.451, it still provided a satisfactory level of accuracy. Overall, Model 2 demonstrated a reasonably good ability to predict ARG dynamics, validating its utility in this context.

3.2. Hydrodynamic Environmental Modeling for Predicting Key Environmental Drivers. The process-based hydrodynamic environmental model demonstrated consistent and reasonable predictive performance across the key parameters: Temp, Zn, DO and LIN, which were identified as four of the five main drivers of ARG_Macro abundance in this study (Table 2 and Figure 4). The overall mean values of key performance metrics across these periods were as follows: for Temp, the model achieved a mean R² of 0.712, an RMSE of 0.569, and an NSE of 0.659; for DO, the mean R² was 0.707, with an RMSE of 0.723 and an NSE of 0.594; Zn predictions yielded a mean R² of 0.737, an RMSE of 0.317, and an NSE of

0.652; and for LIN, the model demonstrated a mean R^2 of 0.761, an RMSE of 0.676, and an NSE of 0.612. These mean values indicate the model's reasonable performance across the different parameters. Focusing on Temp, the model's best predictive performance was observed at location P9, where it recorded an R^2 of 0.832, an RMSE of 0.315, and an NSE of 0.816. This location represents the model's strongest accuracy in capturing temperature variations, suggesting effective alignment between observed and predicted values. The lowest performance occurred in P4, where the R^2 dropped to 0.191, and although the RMSE increased to 1.239, the NSE remained at a reasonable 0.428, indicating that while the correlation was weaker, the model still managed to capture some of the underlying temperature dynamics. For DO, the model demonstrated the highest predictive accuracy in P1, achieving an R^2 of 0.959, an RMSE of 1.448, and an NSE of 0.684. This strong performance reflects the model's ability to simulate dissolved oxygen levels in this place. However, in P6, the model's performance was less robust, with an R^2 of 0.143 and an NSE of 0.234, indicating challenges in accurately predicting DO levels under certain conditions. Despite this, the model still provided a reasonable level of accuracy, as reflected by the NSE value. Zn concentrations were also effectively modeled, with the highest accuracy observed in P9, where the model nearly achieved perfect predictions, recording an R^2 of 0.997, an RMSE of 0.57, and an NSE of 0.875. This indicates a strong model performance in capturing zinc dynamics in this location. The lowest performance for Zn was noted in P5, where the R^2 was 0.257 and the NSE was 0.439, showing that while the model's accuracy was reduced, it still maintained a reasonable predictive capability. LIN predictions were strongest in P8, where the model reached an almost perfect R^2 of 0.998, with an NSE of 0.462, demonstrating its high accuracy in this location. On the other hand, the model's performance in P10 was comparatively lower, with an R^2 of 0.567 and an NSE of 0.712. Despite the lower correlation, the model still managed to provide reliable predictions, as indicated by the NSE. Overall, the process-based hydrodynamic environmental model showed consistent and reasonable predictive performance across all evaluated parameters which affirm the model's utility as a robust tool for providing the reliable (environmental) data set for prediction of ARG_Macro.

The simulated temperature for Singapore coastal waters indicates minimal variation throughout the year, reflecting a stable tropical climate with no significant seasonal changes (Figure S2). Temp values consistently remain above 25 °C, with slightly lower readings from February to April, characteristic of a typical equatorial climate.⁴³ Unlike in temperate regions, where higher temperatures often lead to lower DO levels due to decreased oxygen solubility,⁴⁴ there is no strong inverse relationship between DO levels and temperature in our model results. This suggests that DO levels in Singapore coastal waters are likely influenced by other factors, such as biological activities or anthropogenic inputs,⁴⁵ rather than temperature fluctuations, which remain relatively constant and do not significantly impact DO solubility. The prediction of DO levels does not reveal significant seasonal variation across different points (Figure S3). Spatially, sites in the northern region (P1–P4 and P11–P12) show higher DO levels than those in the southern region (P5–P10). DO concentrations in the northern area are also more variable, likely due to agricultural influences, stronger freshwater inputs, and more dynamic biological activity. The present simulation simplifies

DO dynamics by focusing on water–air exchange (reaeration) processes, which provides reasonable estimates for surface layer oxygenation but does not account for potential oxygen consumption from organic matter mineralization. Future studies incorporating BOD/COD measurements could significantly enhance our understanding by quantifying the actual organic pollution levels across the study area and enabling the simulation of mineralization processes that would more completely represent the coastal DO budget. Understanding these seasonal trends in DO is crucial for assessing aquatic health and identifying periods when marine life may be at risk due to lower DO levels. Additionally, DO levels could serve as a useful surrogate marker for predicting ARG hotspots in aquatic environments.⁴⁶ The prediction of Zn concentrations exhibits a distinct temporal pattern, with the highest concentrations recorded during March and May, across most sampling points (Figure S4). Spatially, the highest concentrations are consistently found at sampling points P1 and P2, with the northern locations, especially P2, P3, and P4, showing a significant presence of Zn. Interestingly, there is a noticeable decline in concentrations during the warmer months of June to August, particularly at points P3 to P10. This data reflects both seasonal variability and spatial distribution of Zn in the studied area, potentially influenced by factors such as monsoon seasons⁴⁷ and agricultural runoff.⁴⁸

The prediction of LIN concentrations indicates notable variability both spatially and temporally (Figure S5). Higher concentrations are observed during March, April and most prominently in August, potentially linked to seasonal activities, particularly the monsoon season, which has been identified as a key factor driving biogeochemical dynamics in Singapore's coastal waters.⁴⁸ The monsoon season brings increased rainfall, which can flush more contaminants from the land into the sea, thereby impacting coastal water quality.⁴⁵ The highest concentrations are consistently found at points P1 and P2. Additionally, predicted results from November and December, for example at points P11 and P12, show a significant spike, which could be indicative of event-based pollution or shifts in environmental conditions that influence LIN levels. Generally, higher levels were predicted at points P1, P2, P3, and P4, identifying these areas as hotspots for LIN. Notably, these hotspots are all located in the Johor Strait, which is a shared coastal water body between Singapore and Malaysia.⁴⁹ This area is not only close to agricultural regions on land⁵⁰ but also hosts a high density of aquaculture activities, such as fish farms,⁵¹ further contributing to the elevated LIN concentrations.

The spatial variations in model performance across monitoring stations primarily reflect boundary condition uncertainties and proximity effects. Stations near domain edges (P5, P10) showed poorer fits due to unresolved boundary exchanges, while northern stations (P4, P5) located at Johor Strait were affected by unquantified cross-border inputs from Malaysia. These limitations underscore the importance of enhanced boundary data collection, particularly through international collaborations to monitor transboundary fluxes. Future model improvements should focus on higher-resolution boundary schemes and expanded monitoring networks to better constrain edge effects, which would significantly benefit regional environmental management and predictive accuracy in coastal zones.

3.3. Hybrid Modeling for Antibiotic Resistance Gene Prediction. The hybrid model, which couples the process-

based hydrodynamic environmental model with a data-driven model, demonstrated a strong and consistent ability to predict ARGs_Macro over time and space (P1–P12). The overall mean performance metrics for the hybrid model indicated an R^2 of 0.693, an RMSE of 0.026, and an NSE of 0.589, suggesting a reasonable balance between accuracy and predictive reliability. The model's highest predictive performance was observed in P2, where it achieved an impressive R^2 of 0.95, an RMSE of 0.03, and an NSE of 0.801, indicating a very strong alignment between observed and predicted ARGs concentrations. Similarly, P3 also showed robust performance, with an R^2 of 0.87 and an NSE of 0.766, further underscoring the model's effectiveness during these periods. Conversely, the hybrid model's lowest performance was recorded in P8, where the R^2 was 0.438 and the NSE was 0.437. Despite this lower accuracy, the model still managed to provide reasonable predictions, as indicated by the NSE value. Additionally, P5 also saw a relatively lower performance, with an R^2 of 0.477 and an NSE of 0.421, although it still maintained a predictive capability above the acceptable threshold. Overall, the hybrid model's performance across the periods was consistent, with all NSE values exceeding 0.3, which is indicative of the model's reliable predictive capacity. This coupling of process-based and data-driven approaches enabled the model to effectively capture the complex dynamics of ARGs, making it a robust tool for environmental predictions.

The predicted temporal variability of ARGs_Macro concentrations across 12 locations reveals distinct patterns linked to monsoonal cycles (Figure S6). The model forecasts higher ARG levels during the NE and SW Monsoon, particularly in January and February, when increased surface runoff from intensive rainfall likely transports pollutants from agricultural and aquaculture activities into nearby water bodies.⁵² Stations such as P1 and P4 were predicted to show elevated concentrations during this period, with peaks reaching up to 0.18 GC 16S RNA. In contrast, the intermonsoon, characterized by drier conditions, is expected to result in relatively lower ARG concentrations, with more stable levels predicted during these months. Short-term spikes at locations like P1, P2, and P3 in January and February may correspond to episodic pollution events which warrants further investigations.

3.4. Hotspots of Antimicrobial Resistance through Holistic Spatiotemporal Model Results. Hotspot maps of ARGs during both the NE and SW monsoon seasons showed that ARG_Macro levels were noticeably higher along the northern and northeastern coasts, in the Johor Strait, a waterway shared between Singapore and Malaysia (Figure 5). Due to the Johor Strait's relatively narrow and shallow nature, monsoonal winds primarily drive the sea currents and surface drift patterns. Exposure to monsoonal winds and increased precipitation during the SW and NE monsoons has led to a notable rise in ARG_Macro levels in these areas, confirming the anticipated bilateral diffusion from various land uses, particularly agriculture and aquaculture activities along the coastline. For instance, the upper northeastern region encompasses Singapore's fish farms, where intensive aquaculture activities occur, alongside other anthropogenic inputs. The runoff from these activities introduces both antibiotics and resistance genes into the surrounding coastal waters, thereby increasing ARGs prevalence.⁵³ Additionally, the nutrient-rich environment, resulting from fertilizer use in agricultural practices, can further promote microbial growth, enhancing the spread of the antibiotic resistome in these regions.⁵⁴

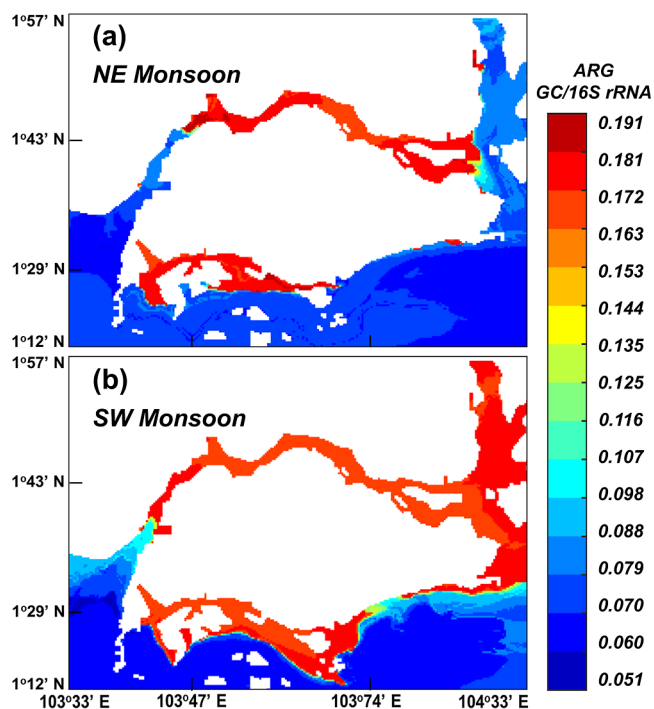


Figure 5. Model-predicted spatial distribution of mean ARG_Macro concentrations during the (a) Northeast Monsoon and (b) Southwest Monsoon seasons.

Interestingly, this study found a significant inverse relationship between DO and ARG_Macro levels. Lower DO levels were correlated with higher ARG_Macro, suggesting that hypoxic conditions may favor the persistence of antibiotic resistance and facilitate the maintenance and transfer of ARGs within microbial communities.⁵⁴ Recent investigations into the antibiotic resistome in tropical rivers in Malaysia also suggest that DO can serve as a surrogate marker for predicting antibiotic resistance in aquatic environments, which is consistent with our predicted findings.⁴⁶ In addition to the hotspots in the Johor Strait, ARG_Macro hotspots are also identified along the southwestern coasts, an industrial area where higher pollutant loads contribute to the spread of the antibiotic resistome. Although antibiotic inputs from industrial sites may be lower than those from agricultural or aquaculture regions, the discharge of heavy metals, such as Zn, from industrial activities likely contributes to the persistence and spread of ARGs. Zn and other metals can coselect for bacteria that harbor both metal resistance and antibiotic resistance genes, facilitating the survival and transfer of ARGs even in environments with lower direct antibiotic exposure.

However, compared to the hotspots during the NE monsoon, the ARG_Macro hotspot regions expand during the SW monsoon, with new hotspots predicted along the southeast coasts and the upper northeastern region near the upstream section of the Johor Strait, known as the Johor River. This distribution pattern is primarily attributed to differences in meteorological forcing between the two monsoon seasons. During the NE monsoon season, monsoonal winds from the northeast drive the flow from upstream of the Johor River toward the sea. The large volume of seawater and strong mixing mechanisms accelerate the dilution of pollutants from the upstream areas. As a result of monsoonal current reversals during the SW monsoon season, ARG_Macro accumulates in

the Johor Strait. Similarly, the southwest-directed sea currents cause pollutant loads from Singapore's southern coast to become blocked and accumulate in the area. The comparison between the NE and SW Monsoons highlights the significant role of seasonal hydrodynamic processes in shaping ARG distributions for these waters. This seasonal variability underscores the necessity of understanding monsoonal dynamics when assessing ARG dispersal and concentration in marine environments. The persistence of high ARG levels nearshore during both monsoons raises concerns regarding the potential risks to coastal water quality and public health. Coastal environments, particularly those influenced by agricultural runoff and aquaculture activities, appear to be vulnerable to ARG contamination, which may contribute to the spread of antibiotic resistance in marine ecosystems. The predicted hotspots emphasize the importance of monitoring and mitigating ARG sources, especially in areas where hydrodynamic conditions facilitate their accumulation.

4. ENVIRONMENTAL IMPLICATIONS

The results of this study carry critical implications for the management of coastal water quality and the control of AMR dissemination. The identification of key environmental drivers, particularly LIN and Zn, as significant factors influencing ARG_Macro levels emphasizes the need for integrated management strategies targeting both antibiotics and heavy metal contamination in coastal regions. The role of Zn, a heavy metal commonly linked to coselection mechanisms for antibiotic resistance genes, highlights the importance of addressing bilateral pollution from industrial and agricultural-derived inputs of antibiotics. The spatiotemporal hotspot prediction revealed that coastal zones, particularly in the Johor Strait, a shallow coastal water body shared between Singapore and Malaysia, are highly susceptible to ARG accumulation, especially during monsoon seasons when hydrodynamic conditions facilitate the concentration and spread of these genes. This seasonal variability points to the necessity of adaptive and targeted monitoring efforts, focusing on periods of increased hydrodynamic activity that may enhance the persistence and distribution of ARGs. Moreover, the inverse relationship between DO levels and ARG_Macro suggests that hypoxic conditions in coastal environments may play a role in sustaining antibiotic resistance, which raises further concerns about the broader ecological impacts of deteriorating water quality on microbial communities and resistance gene proliferation. Ultimately, this study provides valuable insights into the environmental drivers of ARGs in coastal waters and offers a robust modeling framework that can inform both risk management strategies and future research on mitigating the risks of antimicrobial resistance in marine ecosystems. The findings underscore the importance of a multidisciplinary approach to water quality management, integrating environmental monitoring and environmental modeling to inform strategies that protect coastal ecosystems and public health from the escalating threat of antibiotic resistance.

■ ASSOCIATED CONTENT

SI Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/acs.est.5c01927>.

The Supporting Information contains the analytical methods for physicochemical parameters, antibiotics and

antimicrobial resistance genes, as well as a detailed model description, supplemental result tables, and figures (PDF)

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Notes

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