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Research Paper

Antibiotic Resistance Profiling of Hemolytic *Shewanella* Species in Oysters and Seawater from the Mid-Atlantic Region

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ABSTRACT

Shewanella species are opportunistic pathogens and food spoilage bacteria that can be transmitted through contaminated seawater and seafood. Immunocompromised individuals are at greater risk when consuming raw fish and shellfish or when open wounds are exposed to contaminated marine environments. The emergence of antibiotic-resistant bacteria has raised concerns over public health and animal health globally. Infections involving resistant hemolytic bacteria can be life-threatening due to limited treatment options. This study aimed to evaluate the resistance profiles of hemolytic *Shewanella* species against 21 antibiotics commonly used to treat Gram-negative bacterial infections. A total of 166 hemolytic isolates recovered from oyster (n = 107) and seawater (n = 59) samples obtained from the Mid-Atlantic sampling sites between 2019 and 2021 were tested using Sensititre GNX2F antimicrobial susceptibility plates. Overall, 16.27% of oyster isolates and 18.94% of seawater isolates were resistant to one or more antibiotics. Only Ertapenem showed a statistically significant difference in resistance between alpha and beta hemolytic groups. Resistance levels varied across species. *Shewanella algae* and *S. khirikhana* exhibited the highest resistance to 10 and 14 antibiotics, respectively, followed by *S. marisflavi* (11 antibiotics) and *S. indica* (9 antibiotics). Doxycycline, Levofloxacin, Minocycline, and Tigecycline were the most effective antibiotics, with low or no resistance observed among *Shewanella* isolates from both seawater and oysters. This is the first study to provide detailed insights into the antibiotic resistance profiles of hemolytic *Shewanella* species in the Chesapeake Bay and the Maryland Coastal Bays.

Marine and estuarine environments serve as reservoirs for diverse microbial communities, including *Shewanella* species. Traditionally studied for their role in biogeochemical cycles, these Gram-negative, nonfermentative, facultative anaerobes have increasingly been recognized as opportunistic pathogens with clinical significance (Ng et al., 2022; Kang et al., 2024; Sher et al., 2025) particularly in individuals with underlying conditions such as hepatobiliary diseases, malignancy, or immunosuppression (Ng et al., 2022; Yu et al., 2022; Johnson et al., 2025b). *Shewanella* species are widely distributed in marine habitats, including fish, shellfish, seawater, and marine sediments (Wang et al., 2024; Johnson et al., 2025a). Recently, our team investigated the prevalence of *Shewanella* species in oyster and seawater samples collected from the Chesapeake and Maryland Coastal Bays. A total of 1,344 presumptive isolates were screened and confirmed using biochemical tests, hemolytic activity, and 16S rRNA sequencing (Johnson et al., 2025a). The top four species were *S. khirikhana,* a known shrimp pathogen (49%), *S. marisflavi* (19%), *S. loihica* (11%), and *S. algae* (8%). In seawater, 71% of the sequenced isolates were identified as *Shewanella* and the counts ranged from undetectable to 4.0×10^2 CFU/ml. In oyster samples, 65% of the sequenced isolates were *Shewanella,* and the counts ranged from undetectable to 1.80×10^7 CFU/g. Moreover, 45% and 54% of the isolates were beta and alpha hemolytic, respectively.

While generally considered environmental bacteria, certain species, such as *S. algae* and *S. putrefaciens*, have been implicated in human infections, particularly in immunocompromised individuals exposed to contaminated seawater or seafood (Myung et al., 2009; Zong, 2011; Kalathuru et al., 2023; Johnson et al., 2025b). Their ability to cause severe soft tissue and bloodstream infections, coupled with frequent misidentification as *Vibrio* species, complicates diagnosis and treatment (Myung et al., 2009). Unlike other Gram-negative pathogens, such as *Vibrio* and *Aeromonas*, antibiotic resistance in *Shewanella*

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is not systematically monitored, as it is not classified as a reportable pathogen by the CDC, leading to an underestimation of its clinical significance (McAuliffe et al., 2015; Johnson et al., 2025b; Sher et al., 2025).

Although *Shewanella* infections remain relatively rare, their increasing antibiotic resistance presents a growing concern for public health and clinical management. Antibiotic resistance in *Shewanella* has been observed in both clinical and environmental isolates, with resistance to commonly used antimicrobials such as beta-lactams, aminoglycosides, and fluoroquinolones (Von Wintersdorff et al., 2016; Byun et al., 2017; Sher et al., 2025). Recent global studies have identified multidrug resistant (MDR) *Shewanella* strains in diverse settings, including aquaculture, dairy farms, milling environments, and marine habitats, raising concerns about environmental reservoirs of resistance genes (Taherzadeh et al., 2014; Li et al., 2022b; Kang et al., 2024; Sher et al., 2025; Johnson et al., 2025b). The misuse and overuse of antibiotics in human medicine, aquaculture, and agriculture have likely contributed to the persistence and spread of resistant *Shewanella* strains in marine environments (Morina & Franklin, 2023; Johnson et al., 2025b; Sher et al., 2025). Coastal ecosystems, including the Chesapeake Bay and Maryland Coastal Bays, are particularly vulnerable due to urban and agricultural runoff which increases bacterial exposure to antibiotics and other contaminants (University of Maryland Extension, 2025; Maryland Coastal Bays Program, 2025; Sher et al., 2025; Maryland Department of Natural Resources, 2024). This environmental pressure may drive the selection and maintenance of resistant strains in these ecosystems, raising concerns about the potential for transmission to human populations through seafood consumption and recreational water activities.

Despite the emerging recognition of *Shewanella* as a clinically relevant pathogen, studies on its antibiotic resistance profiles remain scarce, particularly in the Mid-Atlantic region. Prior research has largely focused on *S. algae* and *S. putrefaciens*, with limited antibiotic susceptibility testing involving only a small subset of drugs (Byun et al., 2017). Resistance trends in other *Shewanella* species, such as *S. khirikhana, S. marisflavi,* and *S. loihica*, remain largely unexplored despite their prevalence in seafood-associated environments (Kang et al., 2024; Johnson et al., 2025a). To date, no study has systematically assessed antibiotic resistance in *Shewanella* isolates from the Chesapeake Bay and Maryland Coastal Bays. Given the overuse of antibiotics in clinical settings, veterinary medicine, and aquaculture, and as additives to animal feeds, baseline studies are needed to determine MDR in *Shewanella* strains from coastal waters and shellfish.

The objective of this study was to determine the antibiotic resistance profiles of *Shewanella* species recovered from oysters and seawater samples in the Mid-Atlantic region. By assessing resistance patterns across multiple antibiotic classes, this research provides baseline data on *Shewanella* antibiotic susceptibility, informs potential risks to seafood safety, and contributes to broader discussions on the role of environmental reservoirs in antimicrobial resistance.

Materials and methods

Sample collection. Oyster and seawater samples were collected monthly from 2019 to 2021 at three sites in the Mid-Atlantic region: Honga River and Tangier Sound (Chesapeake Bay) and one site within the Maryland Coastal Bays (Johnson et al., 2025a). Detailed protocols for sample collection and initial processing are described in Johnson et al. (2025a). From these samples, a total of 166 *Shewanella* isolates were selected, consisting of 82 alpha-hemolytic and 84 beta-hemolytic isolates. The alpha hemolytic isolates included *S. algae, S. loihica,* and *S. marisflavi*, whereas the beta-hemolytic isolates were primarily *S. algae* and *S. khirikhana* (Johnson et al., 2025a). Alpha hemolytic isolates partially break down hemoglobin, producing a green discoloration on blood agar, whereas beta hemolytic isolates completely lyse red blood cells, forming a clear zone around colonies. Hemolytic activity was assessed using 5% sheep blood agar, following standard laboratory protocols (Johnson et al., 2025a).

Antimicrobial susceptibility testing. A total of 166 *Shewanella* isolates were tested against 21 clinically relevant antibiotics using the Sensititre microbroth dilution method, following Clinical and Laboratory Standards Institute (CLSI) guidelines (CLSI, 2016, 2024). For antibiotic lacking established CLSI breakpoints, susceptibility interpretations were based on U.S. Food and Drug Administration (FDA) guidelines (Table 1). Quality control was ensured using *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853. The minimum

Table 1
Types, ranges of concentrations and interpretive criteria for minimal inhibitory concentration (MIC) for tested antibiotics

Group	Antimicrobial Agent	Abbreviation	Concentrations (μg/mL)	Interpretive Categories & MIC Breakpoints		
				<Susceptible	Intermediate	Resistant>
Aminoglycosides	Amikacin	AMI	4–32	≤16	32	≥64
Beta-Lactam	Aztreonam	AZT	2–16	≤4	8	≥16
Beta-Lactam	Cefepime	FEP	2–16	≤2	4–8	≥16
Beta-Lactam	Cefotaxime	FOT	1–32	≤1	2	≥4
Beta-Lactam	Ceftazidime	TAZ	1–16	≤4	8	≥16
Fluoroquinolone	Ciprofloxacin	CIP	0.25–2	≤1	2	≥4
Polymyxin	[b]Colistin	COL	0.25–4	–	≤2	≥4
Carbapenem	Doripenem	DOR	0.12–2	≤1	2	≥4
Tetracycline	Doxycycline	DOX	2–16	≤4	8	≥16
Carbapenem	Ertapenem	ETP	0.25–4	≤0.5	1	≥2
Aminoglycosides	Gentamicin	GEN	1–8	≤4	8	≥16
Carbapenem	Imipenem	IMI	1–8	≤1	2	≥4
Fluoroquinolone	Levofloxacin	LEVO	1–8	≤2	4	≥8
Carbapenem	Meropenem	MERO	1–8	≤1	2	≥4
Tetracycline	Minocycline	MIN	2–16	≤4	8	≥16
Beta-Lactam	Piperacillin-tazobactam constant 4	P/T4	8/4–64/4	≤16/4	32/4–64/4	≥128/4
Polymyxin	[b]Polymyxin B	POL	0.25–4	–	≤2	≥4
Beta-Lactam	Ticarcillin-clavulanic acid constant 2	TIM2	16/2–128/2	≤16/2	32/2–64/2	≥128/2
Glycylcycline	[a]Tigecycline	TGC	0.25–8	≤2	4	≥8
Aminoglycosides	Tobramycin	TOB	1–8	≤4	8	≥16
Sulfonamide	Trimethoprim-sulfamethoxazole	SXT	0.5/9.5–4/76	≤2/38	–	≥4/76

[a] Used standards recommended by FDA.
[b] Used CLSI M100-A32 (2016) Table 2A for Enterobacteriaceae CLSI M100-ED34 (2024) standards table 1A-1 were used for all antibiotics except for COL, POL, and TGC.

inhibitory concentration (MIC) was determined to be the lowest antibiotic concentration that completely inhibited bacterial growth (CLSI, 2016, 2024). Even though the names and abbreviations of the 21 antibiotics used in this study are abbreviated after first use throughout the text, the names and abbreviations are also provided in Table 1 for easy reference for the reader.

Inoculum preparation and panel inoculation. Sensititre sterile water tubes, Mueller-Hinton broth tubes, and minimum inhibitory concentration (MIC) panels (Sensititre GNX2F; Thermo Fisher Scientific Inc., Waltham, MA, USA) were labeled accordingly. Using an inoculation loop, individual colonies were collected from Tryptic Soy Agar (TSA) plates, and a 0.5 McFarland standard suspension was prepared in sterile water by visual comparison to the McFarland turbidity standard mentioned previously. Fifty microliters (μL) of this suspension was transferred to Mueller-Hinton broth with TES buffer and mixed via vortexing. The inoculum was transferred to a sterile reservoir and dispensed into Sensititre GNX2F plate wells using an 8-channel pipette. The broth was used within 30 min of preparation. Sensititre GNX2F plates were sealed and incubated at 35 °C for 24 h.

Results interpretation and quality control. After incubation, positive control wells were examined for bacterial growth, indicated by turbidity. The susceptibility of 166 isolates to 21 antibiotics was determined for the following species: *S. algae, S. khirikhana, S. marisflavi, S. seohaensis, S. submarina,* and *S. loihica*. Isolates were classified as susceptible, intermediate, or resistant based on established breakpoints (Table 1). Multidrug resistance was defined as nonsusceptibility to at least one agent in three or more antimicrobial categories, according to CLSI, 2016 and 2024 standards.

Statistical analysis. All statistical analyses were performed using RStudio (2024.09.1 + 394), with significance set at $\alpha = 0.05$. Due to nonnormal data distribution, the Kruskal-Wallis test, a nonparametric alternative to one-way ANOVA, was used to assess differences in antibiotic resistance across sampling sites and among *Shewanella* species (Conover, 1999; McDonald, 2014). For antibiotics with significant differences, Dunn's test was used for pairwise comparisons, with Bonferroni corrections applied for multiple testing (Dunn, 1964). The Wilcoxon rank-sum test was applied to compare resistance between sample types (seawater vs oysters) and hemolysin groups (alpha vs beta), as it does not assume normality (Mann & Whitney, 1947; Conover, 1999). Significance was determined using Bonferroni-adjusted p values, with $p < 0.05$ considered as statistically significant.

Results

Antibiotic resistance by sampling sites. Antibiotic resistance varied significantly across the sampling sites in the Chesapeake and Maryland Coastal Bays, based on both oyster and seawater isolates combined (Fig. 1). Each site displayed distinct resistance profiles. At Honga River (Fig. 1A), the highest resistance was observed for Ertapenem (ETP, 47.5%), followed by Polymyxin B (POL, 45%), Colistin (COL, 37.5%), and Meropenem (MERO, 30%). Moderate resistance was observed for Aztreonam (AZT) and Cefotaxime (FOT), each at 25%, while Ticarcillin-clavulanic acid (TIM2) was 17.5% and both Imipenem (IMI) and Piperacillin-tazobactam constant 4 (P/T4) exhibited resistance levels of 15%. Isolates from Honga River ($n = 40$) were susceptible to 11 antibiotics, as shown in Figure 1A. At Horn Point (Fig. 1B), high resistance was seen for POL (39.2%), followed closely by IMI (33.3%), COL (33.3%), TIM2 (31.4%), and P/T4 (31.4%). Both AZT and FOT showed 29.4% resistance. Isolates from Horn Point ($n = 34$) were susceptible to four antibiotics as shown in

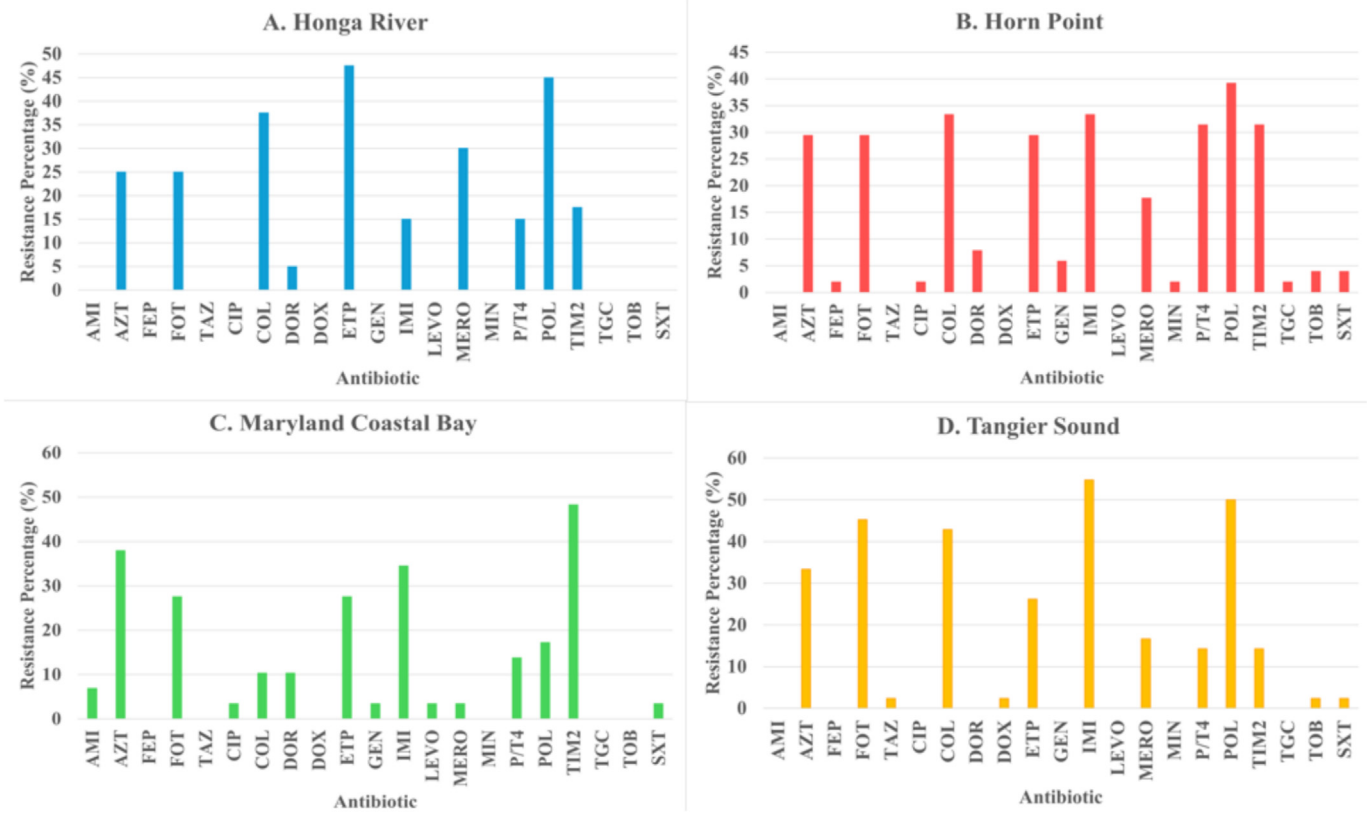

Figure 1. Percent of isolates resistant to antibiotics across four sampling sites. (A) Honga River, (B) Horn Point, (C) Maryland Coastal Bay, and (D) Tangier Sound. Each bar represents the percentage of isolates resistant to each antibiotic. Abbreviations for antibiotics used in this study are as follows: AMI (Amikacin), AZT (Aztreonam), FEP (Cefepime), FOT (Cefotaxime), TAZ (Ceftazidime), CIP (Ciprofloxacin), COL (Colistin), DOR (Doripenem), DOX (Doxycycline), ETP (Ertapenem), GEN (Gentamicin), IMI (Imipenem), LEVO (Levofloxacin), MERO (Meropenem), MIN (Minocycline), P/T4 (Piperacillin-tazobactam constant 4), POL (Polymyxin B), TIM2 (Ticarcillin-clavulanic acid constant 2), TGC (Tigecycline), TOB (Tobramycin), and SXT (Trimethoprim-sulfamethoxazole).

Figure 1B. In Maryland Coastal Bay (Fig. 1C), the highest resistance was observed for TIM2 (48.3%), followed by AZT (37.9%), and IMI (34.5%). Resistance to ETP and FOT was 27.6% and POL was 17.2%, while COL remained relatively low at 10.3%. Isolates from Maryland Coastal Bay were susceptible to 6 antibiotics, as shown in Figure 1C, but were resistant to TIM2 (48.3%), AZT (38%), IMI (34.5%), and almost equally for FOT (27.6%) and ETP (27.6%). At Tangier Sound (Fig. 1D), IMI showed the highest resistance at 54.3%, followed by POL (50%), FOT (45.2%), and AZT (33.3%). Resistance to ETP was 26.2%, while TIM2 showed resistance at 14.3%. The lowest resistance was observed for Doxycycline (DOX, 2.4%) and Ceftazidime (TAZ, 2.4%), Tobramycin (TOB, 2.4%), and Trimethoprim-sulfamethoxazole (SXT, 2.4%). At Tangier Sound ($n = 63$), isolates were susceptible to eight antibiotics as shown in Figure 1D.

Kruskal-Wallis tests revealed significant differences in resistance across sampling sites for six antibiotics: Amikacin (AMI), COL, IMI, MERO, POL, and TIM2 ($p < 0.05$). Posthoc Dunn's tests identified significant pairwise differences for IMI between Honga River and Tangier Sound ($p < 0.001$), and for TIM2 between Honga River and Tangier Sound ($p < 0.001$). Additionally, MERO and POL showed significant differences between Honga River and Maryland Coastal Bay ($p < 0.05$), and between Maryland Coastal Bay and Tangier Sound ($p < 0.05$). These analyses reflect site-specific variations in resistance among *Shewanella* isolates recovered from both oyster and seawater samples.

Antibiotic resistance by hemolysin. Resistance patterns varied between alpha- and beta-hemolytic isolates (Fig. 2). Data from both oyster and seawater isolates were pooled for this analysis to provide a comprehensive overview of *Shewanella* resistance profiles by hemolysin type. Among alpha-hemolytic isolates ($n = 82$) (Fig. 2, blue bars), the highest resistance was observed for AZT (32.9%), followed by POL and TIM2 both at 31.7%, respectively, FOT (30.5%), and IMI (29.3%). Moderate resistance levels were seen for COL (25.6%) and ETP (24.4%). Minimal resistance (<2%) was for Levofloxacin (LEVO), Minocycline (MIN), and Tigecycline (TGC). Resistance to most other antibiotics was minimal (Fig. 2), and none of the isolates were sensitive to Cefepime (FEP), TAZ, or DOX.

Among beta-hemolysin isolates ($n = 80$), resistance levels were generally higher (Fig. 2, red bars). The highest resistance was recorded for POL (47.5%), followed by IMI (40.5%), ETP (41.2%), COL (40%), FOT (33.8%), and AZT (28.6%). Minimal resistance (<2.5%) was detected for DOX, TAZ, FEP, TOB, and SXT. None of the beta-hemolytic strains were sensitive to AMI, LEVO, MIN, or TGC.

The Wilcoxon rank-sum test revealed a significant difference in ETP resistance between alpha and beta hemolytic isolates ($p = 0.0404$), with higher resistance in the beta-hemolytic isolates. No significant differences were observed for other antibiotics tested (all $p > 0.05$).

Antibiotic resistance by sample type. Resistance patterns differed between seawater and oyster samples (Fig. 3). Among seawater isolates, the highest resistance was observed for IMI (55.2%), followed by FOT (37.9%), AZT (36.2%), POL (35.6%), and COL (35.9%). Moderate resistance was observed for ETP (20.7%) and MERO (15.5%). Minimal resistance (1.7%) was detected for AMI, FEP, and MIN. None of the seawater isolates ($n = 58$) were sensitive to Ciprofloxacin (CIP), FEP, LEVO, or MIN, suggesting these antibiotics might be valid treatment options.

In contrast, oyster isolates exhibited a different resistance profile. The highest resistance was observed for POL (40.6%), followed by ETP (39.6%), COL (31.7%), FOT (29.7%), TIM2 (29.7%), AZT (28.7%), and MERO (19.8%). Minimal resistance (2.5%) was observed for AMI and CIP, respectively. Among oyster isolates ($n = 108$), no resistance was detected with DOX, TAZ, TGC, or TOB, suggesting that these may be useful for treating shellfish-associated infections.

The Wilcoxon rank-sum tests revealed significant differences in resistance for ETP and IMI. Resistance to ETP was significantly higher in oyster samples compared to seawater samples ($p = 0.0066$), while resistance to IMI was significantly higher in seawater samples compared to oyster samples ($p < 0.0001$). However, no statistically significant differences were observed for other antibiotics (all $p \geq 0.05$).

Antibiotic resistance profiles of *Shewanella* species. Results from the MDR profiles varied significantly among *Shewanella* species (Table 2). The most commonly detected human pathogenic strain of *Shewanella* is *S. algae* which exhibited the broadest resistance profile,

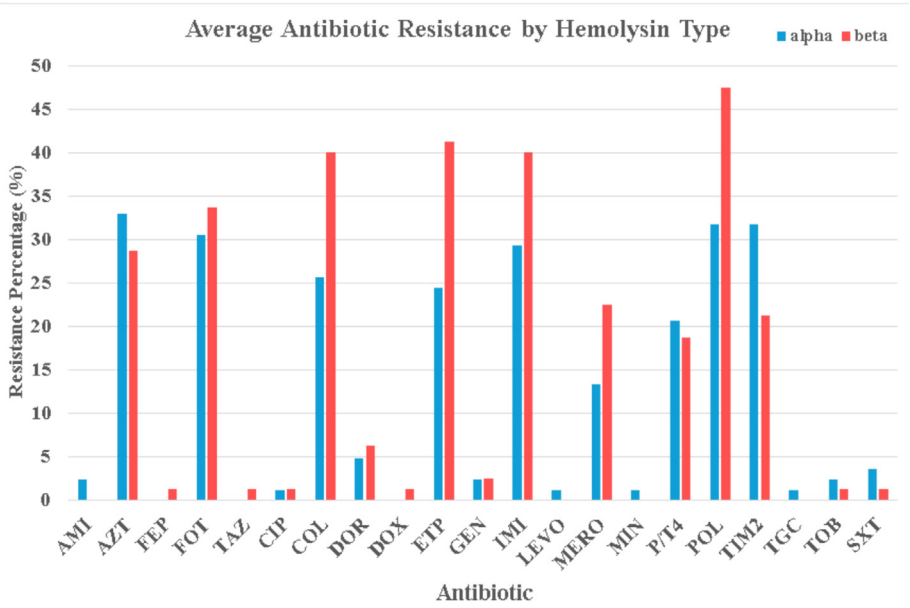

Figure 2. Antibiotic resistance profiles of isolates from seawater and oysters for alpha- and beta-hemolysin groups. Resistance levels to various antibiotics are displayed for isolates from the alpha-hemolysin group (blue bars) and beta-hemolysin group (red bars). Abbreviations for antibiotics used in this study are as follows: AMI (Amikacin), AZT (Aztreonam), FEP (Cefepime), FOT (Cefotaxime), TAZ (Ceftazidime), CIP (Ciprofloxacin), COL (Colistin), DOR (Doripenem), DOX (Doxycycline), ETP (Ertapenem), GEN (Gentamicin), IMI (Imipenem), LEVO (Levofloxacin), MERO (Meropenem), MIN (Minocycline), P/T4 (Piperacillin-tazobactam constant 4), POL (Polymyxin B), TIM2 (Ticarcillin-clavulanic acid constant 2), TGC (Tigecycline), TOB (Tobramycin), and SXT (Trimethoprim-sulfamethoxazole). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

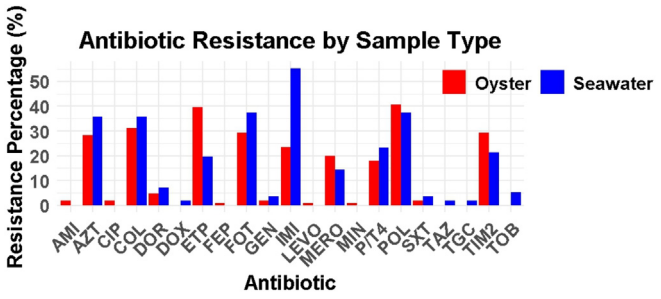

Figure 3. Antibiotic resistance profiles of isolates from oyster and seawater. Resistance levels to various antibiotics are displayed for isolates from seawater (blue bars) and oyster (red bars). Abbreviations for antibiotics used in this study are as follows: AMI (Amikacin), AZT (Aztreonam), FEP (Cefepime), FOT (Cefotaxime), TAZ (Ceftazidime), CIP (Ciprofloxacin), COL (Colistin), DOR (Doripenem), DOX (Doxycycline), ETP (Ertapenem), GEN (Gentamicin), IMI (Imipenem), LEVO (Levofloxacin), MERO (Meropenem), MIN (Minocycline), P/T4 (Piperacillin-tazobactam constant 4), POL (Polymyxin B), TIM2 (Ticarcillin-clavulanic acid constant 2), TGC (Tigecycline), TOB (Tobramycin), and SXT (Trimethoprim-sulfamethoxazole). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

with 26.09% of isolates falling into the "Other" category, which included fewer common combinations of resistance. The most frequently observed MDR profile for *S. algae* was AZT-COL-FOT-IMI-POL (10.87%), followed by AZT-COL-FOT-ETP-IMI-POL-TIM2, AZT-COL-FOT-POL-TIM2, and AZT-FOT-IMI-POL (each occurring at 6.52%, respectively). These patterns indicate substantial resistance to beta-lactams, carbapenems, colistin, and polymyxins. Similarly, the shrimp pathogen *S. khirikhana* also displayed diverse MDR profiles, with 31.91% of isolates categorized as "Other". The most prevalent MDR profile was COL-ETP-FOT-MERO-P/T4-POL (8.51%), followed by COL-ETP-POL (8.51%) and COL-ETP-MERO-POL (6.38%). These profiles highlight substantial resistance to carbapenems, cephalosporins, and polymyxins. *Shewanella algae* (n = 46) and *S. khirikhana* (n = 47) were not susceptible to FEP, suggesting that this antibiotic may still be effective against these two species despite their multidrug-resistant profiles.

Other species also showed unique resistance combinations (Table 2). For example, *S. indica* had profiles such as AMI-AZT-CIP-ETP-GEN-P/T4-SXT-TIM2 and AMI-AZT-P/T4-TIM2 (each 20%). *Shewanella loihica* most frequently exhibited AZT-IMI-P/T4-TIM2 (13.3%) among a broader "Other" category (66.7%). *Shewanella marisflavi* had unique combinations like AZT-DOX-FOT-GEN-IMI-MERO-P/T4-SXT-TIM2-TOB, though most isolates (85%) were categorized as "Other". *Shewanella amazonensis*, while rarely reported in clinical cases, had 62.5% of isolates in the "Other" category, and 25% expressed the COL-ETP-MERO-POL MDR profile. *Shewanella indica* (n = 5) showed 100% susceptibility to nine antibiotics, including FEP, FOT, and MERO. *Shewanella loihica* (n = 15) was fully susceptible to 17 antibiotics, while *S. marisflavi* (n = 20) showed complete susceptibility to AMI, IMI, MERO, and P/T4. *Shewanella amazonensis* (n = 8) exhibited 100% susceptibility to six antibiotics, including CIP, DOX, and LEVO.

Notably, *S. xiamenensis*, a species documented in human clinical infections, demonstrated the least multidrug resistance among all species analyzed. Only one isolate (10%) exhibited resistance to COL-ETP-POL, while the remaining 90% were not classified under any MDR pattern, reinforcing its relatively low resistance profile in

Table 2
Shewanella multidrug resistance (MDR) profiles

Species	MDR Combination[b]	Count[c]	Percentage[d]
S. algae (n = 46)[a]	Other	12	26.09%
	AZT-COL-FOT-IMI-POL[e]	5	10.87%
	AZT-COL-FOT-ETP-IMI-POL-TIM2	3	6.52%
	AZT-COL-FOT-POL-TIM2	3	6.52%
	COL-FOT-IMI-POL	3	6.52%
	AZT-COL-FOT-IMI-POL-TIM2	2	4.35%
	AZT-COL-IMI-POL-TIM2	2	4.35%
	AZT-FOT-IMI-POL-TIM2	2	4.35%
S. amazonensis (n = 8)	Other	5	62.50%
	COL-ETP-MERO-POL	2	25%
S. indica (n = 5)	AMI-AZT-CIP-ETP-GEN-P/T4-SXT-TIM2	1	20%
	AMI-AZT-P/T4-TIM2	1	20%
S. khirikhana (n = 47)	Other	15	31.91%
	COL-ETP-FOT-MERO-P/T4-POL	4	8.51%
	COL-ETP-POL	4	8.51%
	COL-ETP-MERO-POL	3	6.38%
	COL-ETP-FOT-MERO-POL	2	4.26%
	ETP-FOT-MERO	2	4.26%
	IMI-P/T4-TIM2	2	4.26%
S. loihica (n = 15)	AZT-IMI-P/T4-TIM2	3	20%
	FOT-IMI-P/T4-TIM2	1	6.67%
S. marisflavi (n = 20)	AZT-DOX-FOT-IMI-MERO-P/T4-SXT-TIM2-TOB	1	5%
	AZT-FOT-GEN-IMI-MERO-P/T4-TIM2-TOB	1	5%
	ETP-P/T4-SXT-TIM2	1	5%
S. submarina (n = 2)	AZT-FOT-SXT	1	50%
S. xiamenensis (n = 10)	COL-ETP-POL	1	10%

[a] n = total number of isolates tested for that species.
[b] MDR profile indicates the combination of antibiotics to which an isolate was resistant. Only the most frequent profiles are listed per species; the "Other" category includes all combinations observed in only one isolate, except for *S. indica, S. loihica, S. marisflavi, S. submarina,* and *S. xiamenensis*, due to their low number of MDR profiles and/or small sample sizes.
[c] Count column represents the number of isolates from that species with that exact MDR profile.
[d] Percentage of MDR isolates within the species exhibiting that specific profile.
[e] Abbreviations for antibiotics: AMI (Amikacin), AZT (Aztreonam), FOT (Cefotaxime), CIP (Ciprofloxacin), COL (Colistin), DOX (Doxycycline), ETP (Ertapenem), GEN (Gentamicin), IMI (Imipenem), MERO (Meropenem), P/T4 (Piperacillin-tazobactam constant 4), POL (Polymyxin B), TIM2 (Ticarcillin-clavulanic acid constant 2), TOB (Tobramycin), and SXT (Trimethoprim-sulfamethoxazole).

this study. *Shewanella xiamaenensis* ($n = 10$) showed complete susceptibility to 11 antibiotics, including gentamicin (GEN), TOB, and TGC, reinforcing its relatively low resistance profile observed in this study.

Dunn's test (Bonferroni-adjusted) identified significant differences among species for multiple antibiotics. For example, FOT resistance varied significantly between *S. algae* and *S. khirikhana* ($Z = 4.69$, adjusted $p = 0.00013$). Similarly, MERO resistance showed significant differences between *S. algae* and *S. khirikhana* ($Z = -5.74$, adjusted $p < 0.0001$). However, other species pairs often did not exhibit significant differences after Bonferroni correction.

Discussion

Antibiotic resistance in *Shewanella* isolates varied significantly across the Chesapeake and Maryland Coastal Bays, likely influenced by anthropogenic activities such as agricultural runoff, wastewater discharge, and aquaculture. For example, the Horn Point site is located adjacent to the Choptank River watershed, which supports over 60,000 acres of cropland and more than 150 permitted animal feeding operations (Maryland Department of Agriculture, 2023). Similarly, the Maryland Coastal Bays hosts over 50 commercial aquaculture operations and receives effluent from multiple wastewater treatment facilities (Maryland Department of Natural Resources, 2024; University of Maryland Extension, 2025). In 2023 alone, the Maryland oyster aquaculture industry reported a record harvest of 94,286 bushels, with 478 shellfish leases covering 7,579 acres of state waterways (Maryland Department of Natural Resources, 2024). Historical water quality profiles from both regions have documented elevated nutrient and antibiotic residues, with measured concentrations of beta-lactam antibiotics ranging from 0.02 to 0.15 μg/L over the past decade (Teng et al., 2019; Morina & Franklin, 2023).

Sites near these agricultural and aquaculture operations, such as Horn Point and Maryland Coastal Bay, showed high resistance to beta-lactams like TIM2 and FOT, consistent with reports identifying estuarine environments as reservoirs for beta-lactam-resistant bacteria (Teng et al., 2019; Morina & Franklin, 2023). In contrast, Honga River and Tangier Sound demonstrated elevated resistance to ETP and POL, which may be linked to industrial discharge from local seafood processing plants and aquaculture facilities, as well as documented antibiotic inputs from these sources (Kang et al., 2024; University of Maryland Extension, 2025). Notably, persistent detection of carbapenem and polymyxin residues in these waterways has been reported in annual monitoring reports since 2015 (Schroeder et al., 2017; Maryland Coastal Bays Program, 2025). The persistence of carbapenem-resistant isolates in these areas is concerning, as even subinhibitory antibiotic concentrations can promote long-term resistance development (Schroeder et al., 2017).

Differences in resistance patterns were also evident between alpha- and beta-hemolytic *Shewanella* isolates. Beta-hemolytic isolates consistently exhibited higher resistance across multiple antibiotic classes, particularly carbapenems and polymyxins. This aligns with studies linking polymyxin resistance to membrane destabilization and lipid scrambling mechanisms in aquatic environments (Fu et al., 2022). Conversely, alpha-hemolytic isolates were generally more susceptible, even though moderate resistance to tetracyclines was detected, consistent with previous reports indicating a decline in tetracycline efficacy against *Shewanella* isolates from environmental and clinical settings (Kang et al., 2024).

A comparison of oyster and seawater samples revealed distinct resistance profiles aligning with recent findings on antibiotic resistance in marine environments. *Shewanella* isolates from seawater samples exhibited the highest resistance to IMI, 55.4%, followed by FOT, 37.5% and AZT, 35.7%, while oyster isolates had higher resistance to POL, 40.2% and ETP, 39.3%. These patterns support the hypothesis that oysters may bioaccumulate resistant bacteria from their environment through filter feeding (Teng et al., 2019). Our findings on carbapenem resistance in seawater isolates (55.4% for IMI) are notably higher than the 5% reported for *Vibrio* spp. in a recent meta-analysis of marine bivalves (Albini et al., 2022), suggesting potential regional variations or species-specific resistance patterns.

The high resistance to POL in oyster isolates (40.2%) aligns with recent studies on aquatic bacteria, where resistance rates of up to 76.9% were observed in clinical isolates (Li et al., 2022a). This trend is concerning given the increasing reliance on polymyxins as last-resort antibiotics. Wilcoxon rank-sum tests further confirmed significant differences in resistance for IMI and ETP between the sample types ($p < 0.0001$ and $p = 0.0066$, respectively). This underscores the potential for seafood-associated bacteria to harbor antibiotic resistance, reinforcing the need for continued monitoring of seafood safety (Ng et al., 2022; Sher et al., 2025).

MDR profiles varied significantly across *Shewanella* species, with *S. algae*, the most isolated species, showing the greatest diversity in MDR combinations, with resistance commonly observed to AZT, COL, FOT, IMI, and POL. Likewise, *S. khirikhana*, a known shrimp pathogen, exhibited resistance to multiple cephalosporins, carbapenems, and polymyxins, suggesting adaptation to highly selective environments (Briant et al., 2020). Less studied species, such as *S. marisflavi* and *S. loihica*, also displayed unique MDR patterns, indicating potential niche-specific adaptations. Given their ability to thrive in highly dynamic environments, *Shewanella* species represent a growing concern for both environmental and public health, warranting continued genomic surveillance and antimicrobial resistance monitoring.

Notably, *S. algae*, the most common pathogenic species in this study, was susceptible to a broad range of antibiotics, including aminoglycosides such as AMI and GEN, as well as fluoroquinolones like LEVO and CIP, indicating potential therapeutic options despite its resistance to certain beta-lactams and carbapenems. In contrast, *S. xiamenensis,* a documented human pathogenic species, demonstrated high susceptibility to most antibiotics tested, with 100% of isolates susceptible to aminoglycosides such as AMI, GEN, and TOB; fluoroquinolones including CIP and LEVO; and beta-lactams such as FEP, TAZ, and FOT, suggesting that this species may pose lower immediate risk and could inform future treatment strategies.

While multidrug resistance was prominent among several *Shewanella* species, patterns of susceptibility revealed potential avenues for therapeutic intervention. Across all isolates tested, DOX, LEVO, MIN, and TGC consistently showed low or no resistance, with DOX exhibiting complete susceptibility in oyster isolates and all isolates from Maryland Coastal Bay and Horn Point. Among alpha-hemolytic isolates, full susceptibility was observed for FEP, TAZ, and DOX, while beta-hemolytic isolates remained fully susceptible to LEVO, MIN, and TGC. Notably, *S. xiamenensis, S. marisflavi,* and *S. loihica* had the highest number of isolates fully susceptible to single antibiotics, with *S. xiamenensis* showing 100% susceptibility to aminoglycosides and fluoroquinolones. These findings highlight the continued efficacy of select antibiotic classes and support their consideration for future treatment and mitigation strategies involving *Shewanella*-related infections and contamination.

Conclusion

The findings presented in this paper provide critical insights into the antibiotic resistance profiles of *Shewanella* species isolated from oysters and seawater in the Chesapeake and Maryland Coastal Bays. These data offer valuable guidance for determining potential treatment scenarios by identifying antibiotics that remain effective against key species such as *S. xiamenensis* and *S. algae*. The widespread resistance observed to clinically significant antibiotics, including carbapenems, colistin, and beta-lactams, raises concerns about the persistence of these pathogens in estuarine environments and their potential impact on public health. Importantly, this study underscores the need for continued surveillance of antimicrobial resistance in marine

ecosystems and supports the development of targeted mitigation strategies. Moreover, the indiscriminate use of antibiotics in aquaculture and livestock production may accelerate resistance among environmental bacteria like *Shewanella,* further complicating treatment options. These results highlight the importance of integrated multidisciplinary approaches to monitor resistance, inform policy, and safeguard both environmental and human health.

CRediT authorship contribution statement

Tahirah Johnson: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Methodology, Investigation, Formal analysis, Data curation. **Trenton Collins:** Writing – original draft, Investigation, Formal analysis. **Gary P. Richards:** Writing – review & editing, Visualization, Validation, Methodology, Funding acquisition, Data curation. **Salina Parveen:** Writing – review & editing, Writing – original draft, Validation, Supervision, Resources, Project administration, Methodology, Funding acquisition, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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