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Antimicrobial Susceptibilities of *Vibrio parahaemolyticus* and *Vibrio vulnificus* Isolates from Louisiana Gulf and Retail Raw Oysters[▽]

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The antimicrobial susceptibilities of 168 *Vibrio parahaemolyticus* and 151 *Vibrio vulnificus* isolates recovered from 82 Louisiana Gulf and retail oysters in 2005 and 2006 were determined. Overall, the two vibrios remained susceptible to the majority of antimicrobials tested; reduced susceptibility was detected only in *V. parahaemolyticus* for ampicillin (81%; MIC ≥ 16 μg/ml). Additionally, *V. parahaemolyticus* displayed significantly higher MICs for cefotaxime, ciprofloxacin, and tetracycline than *V. vulnificus*.

Pathogenic *Vibrio* spp., primarily *Vibrio parahaemolyticus* and *Vibrio vulnificus*, are a leading cause of seafood-associated illness and death in the United States (22, 24). Alarmingly, the incidence of these *Vibrio* infections due to eating raw or undercooked oysters has shown a sustained increase since 2000, as reported recently by FoodNet from the Centers for Disease Control and Prevention (CDC) (4). Tetracycline has been recommended as the antimicrobial of choice for treatment of severe *Vibrio* infections (23), and alternative treatments are combinations of expanded-spectrum cephalosporins (e.g., ceftazidime) and doxycycline or a fluoroquinolone alone (28). Trimethoprim-sulfamethoxazole plus an aminoglycoside is used to treat children in whom doxycycline and fluoroquinolones are contraindicated (6).

Traditionally, *Vibrio* is considered highly susceptible to virtually all antimicrobials (24). During the past few decades, however, antimicrobial resistance has emerged and evolved in many bacterial genera due to the excessive use of antimicrobials in human, agriculture, and aquaculture systems (3, 21). *Campylobacter* and *Salmonella*, two major food-borne pathogens of terrestrial sources, have been studied extensively for the development and dissemination of antimicrobial resistance (7, 14, 15, 26). In contrast, the awareness of antimicrobial-resistant bacteria in the aquatic environment is less well documented (3). The only extensive investigation of antimicrobial susceptibility in *V. parahaemolyticus* in the United States occurred in 1978, even before *V. vulnificus* was first recognized as a food-borne pathogen (2, 18). The few recent studies that examined the antimicrobial susceptibilities of non-*cholerae* *Vibrio* spp. and other aquatic bacteria were all conducted in other countries and included very limited numbers of either *V. parahaemolyticus* or *V. vulnificus* isolates (1, 20, 25, 29, 30). Therefore, the present study aimed to provide an update on the antimicrobial susceptibilities of these two important vibrios by using isolates from Louisiana-harvested raw oysters.

Vibrio was isolated from 82 (87.2%) of 94 oyster samples collected from the Louisiana Gulf Coast ($n = 20$) and retail seafood markets and restaurants ($n = 74$) in Baton Rouge, LA, between June 2005 and September 2006. Oysters shucked onsite in the restaurants or collected from the gulf and the seafood markets in their shells were transported on ice to the laboratory and analyzed within 4 h of collection. Bacterial medium formulations and procedures described in the Food and Drug Administration Bacteriological Analytical Manual (19) and a previous study (13) were used for isolation by both direct plating and enrichment. Following isolation, PCR primers described in the Bacterial Analytical Manual were used for confirmation and examination for the presence of either the *tdh* (coding for thermostable direct hemolysin) or *trh* (coding for Tdh-related hemolysin) gene in *V. parahaemolyticus* (19).

Approximately 60% of the presumptive *Vibrio* isolates were confirmed to be either *V. parahaemolyticus* ($n = 252$) or *V. vulnificus* ($n = 370$), resulting in a total of 622 *Vibrio* isolates. However, none of the 252 *V. parahaemolyticus* isolates possessed Tdh or Trh, which is supported by previous studies indicating low prevalences (0 to 6%) of pathogenic *V. parahaemolyticus* in the United States (10, 11, 12). Interestingly, a much higher detection rate (21.8%) was reported in a recent study in which 48 colonies were examined, particularly during cold months (13). Our study, however, picked only 10 colonies for further analysis, so the zero value for prevalence of pathogenic *V. parahaemolyticus* reported here should be interpreted with caution.

A randomly selected subset of 319 (51.3%) *Vibrio* isolates was examined for susceptibilities to ampicillin, cefotaxime, ceftazidime, chloramphenicol, ciprofloxacin, gentamicin, imipenem, and tetracycline by the broth microdilution method as recommended by the Clinical and Laboratory Standards Institute (CLSI [formerly NCCLS]) (8, 9). Prior to May 2006, no standardized antimicrobial susceptibility testing method was available for non-*cholerae* *Vibrio* spp. The recently published CLSI M45-A document presented the most current information for drug selection, interpretation, and quality control for

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TABLE 1. Antimicrobial susceptibility testing ranges and breakpoints for eight antimicrobials tested and MIC distributions for 319 *Vibrio parahaemolyticus* and *Vibrio vulnificus* isolates recovered from the Louisiana Gulf Coast and retail outlets in 2005 and 2006

Antimicrobial agent	Test range (μg/ml)	Breakpoint (μg/ml)[a]			MIC (μg/ml) distribution for:							
					V. parahaemolyticus (n = 168)				*V. vulnificus* (n = 151)			
		S	I	R	MIC_{50}	MIC_{90}	Range	Mean[b]	MIC_{50}	MIC_{90}	Range	Mean[b]
Ampicillin	0.03–64	≤8	16	≥32	32	32	0.5–64	4.25[c]	1	1	0.06–8	−0.20[c]
Cefotaxime	0.03–64	≤8	16–32	≥64	0.25	0.5	≤0.03–4	−1.85[c]	0.25	0.25	≤0.03–2	−2.27[c]
Ceftazidime	0.03–64	≤8	16	≥32	0.25	0.5	≤0.03–4	−2.13	0.25	0.5	≤0.03–1	−2.17
Chloramphenicol	1–1,024	≤8	16	≥32	1	1	0.06–8	−0.75	1	1	0.06–8	−0.50
Ciprofloxacin	0.03–64	≤1	2	≥4	0.125	0.5	≤0.03–1	−3.28[c]	≤0.03	0.06	≤0.03–0.25	−5.39[c]
Gentamicin	0.06–64	≤4	8	≥16	0.5	2	0.125–2	−0.49	0.5	1	≤0.03–2	−0.57
Imipenem	0.03–64	≤4	8	≥16	≤0.03	0.06	≤0.03–2	−5.29[c]	0.125	0.25	≤0.03–0.5	−3.28[c]
Tetracycline	0.03–64	≤4	8	≥16	0.5	1	0.06–2	−1.14[c]	0.25	0.5	0.06–2	−2.21[c]

[a] Breakpoints recommended by the Clinical and Laboratory Standards Institute in M45-A (8). S, I, and R stand for susceptible, intermediate, and resistant, respectively.
[b] Mean MICs are expressed on a $\log_2$ scale. When the MIC was ≤0.03 μg/ml, −6 was used as the value for mean calculation.
[c] Indicates significantly different mean MICs (in $\log_2$ scale) between *V. parahaemolyticus* and *V. vulnificus* ($P < 0.0001$).

MIC testing of infrequently isolated or fastidious bacteria, including non-*cholerae Vibrio* spp. (8, 17). All eight antimicrobials chosen in this study were in accordance with the M45-A guidelines and represent antimicrobials of clinical importance to non-*cholerae Vibrio* spp., particularly tetracycline, cefotaxime, ceftazidime, and fluoroquinolones.

Table 1 presents the MIC distributions for the 319 *Vibrio* isolates. The MIC_{50}, MIC_{90}, and MIC range for *V. parahaemolyticus* tended to be 1 or more dilutions higher than those for *V. vulnificus*, particularly for ampicillin, where 32- to 64-fold differences were observed. Noticeably, the ampicillin MIC_{50} for *V. parahaemolyticus* fell into the resistance end of the MIC range, whereas that of *V. vulnificus* fell into the susceptibility end. When MICs (expressed on a $\log_2$ scale) sorted by species were analyzed statistically using analysis of variance (SAS for Windows, version 9; SAS Institute Inc., Cary, NC), significant differences were observed for ampicillin, cefotaxime, ciprofloxacin, imipenem, and tetracycline ($P < 0.0001$), among which imipenem was the only one that had a higher mean $\log_2$ MIC for *V. vulnificus* than for *V. parahaemolyticus* (Table 1).

Based on the CLSI-recommended breakpoints (8), the only nonsusceptible isolates identified were 95 ampicillin-resistant and 41 ampicillin-intermediate ones, all being *V. parahaemolyticus*. Therefore, approximately 81% of the 168 *V. parahaemolyticus* tested had ampicillin MICs of ≥16 μg/ml. The 151 *V. vulnificus* isolates, on the other hand, were all susceptible to ampicillin. The results indicated that first-line drugs, including tetracycline, cefotaxime, ceftazidime, and fluoroquinolones, remained highly effective against both *Vibrio* spp.; however, in contrast to recommendations posted by the CDC (5), ampicillin should not be used empirically to treat *V. parahaemolyticus* infection. Interestingly, ampicillin resistance in *V. parahaemolyticus* is not a new phenomenon. A 1978 study in the United States reported that over 90% of 160 *V. parahaemolyticus* isolates were resistant to ampicillin and exhibited β-lactamase activity (18). Multiple studies conducted in other countries since then also reported ampicillin-resistant *V. parahaemolyticus* or *V. vulnificus* isolates in the range of 40 to 100%, although very limited numbers of strains from either species were used (1, 20, 29, 30).

This study represents a large-scale examination of the antimicrobial susceptibilities of both *V. parahaemolyticus* and *V. vulnificus* in Louisiana Gulf and retail oysters. Aquatic bacteria, including vibrios, live in the coastal and estuarine waters, an open area particularly subject to environmental contaminations by agricultural runoff or wastewater treatment plants, which may contain various levels of antimicrobials and heavy metals and act as selective pressure for antimicrobial-resistant aquatic bacteria (16, 27). Despite their public-health significance, strains of *V. parahaemolyticus* and *V. vulnificus* have not been extensively monitored for antimicrobial resistance, in contrast to enteric pathogens such as *Salmonella* or *Campylobacter*. Our findings indicated that the two vibrios remained susceptible to the majority of antimicrobials tested; however, the observed high percentage of *V. parahaemolyticus* isolates with reduced susceptibilities to ampicillin suggests that ampicillin has a potentially low efficiency in empirical treatment of *V. parahaemolyticus* infections. Additionally, discernible differences between these two species in response to antimicrobials were observed, indicating that microbial physiology may play a role. Very recently, studies conducted in South Carolina identified highly resistant strains of *V. parahaemolyticus* and *V. vulnificus* (from estuarine waters, both polluted and pristine, in South Carolina) (Craig Baker-Austin and James D. Oliver, personal communication). Therefore, continued monitoring of both the prevalence and the antimicrobial susceptibility profile is important to better ensure oyster safety; particularly, the retail survey could be expanded to the national level.

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