Occurrence and Antimicrobial Susceptibilities of Pathogenic Vibrios Isolated from Green Mussel, *Perna viridis* L. 1758 in Bacoor bay, Cavite

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Pathogenic vibrios

- Primarily involved in causing gastrointestinal illnesses, wound infection and bacteremia in humans (Forbes *et al*. 2002).
- Have been implicated in water- and seafood-related outbreaks of gastrointestinal infections also in humans (Oliver and Kaper 1997).
- Include:
  - *V. cholerae, Vibrio parahaemolyticus*, *V. vulnificus*, *V. mimicus*, *V. alginolyticus*, and *V. hollisae*

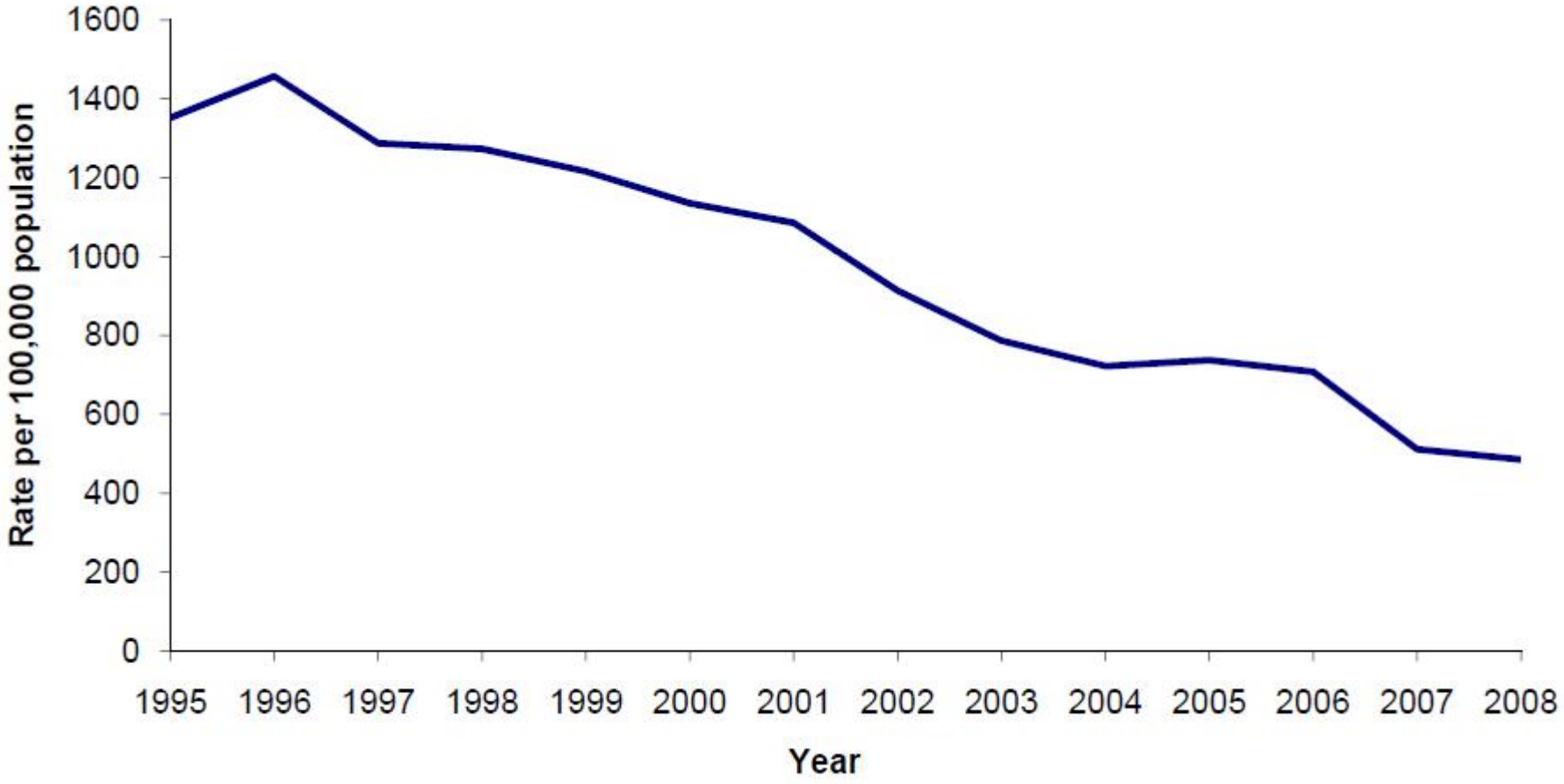
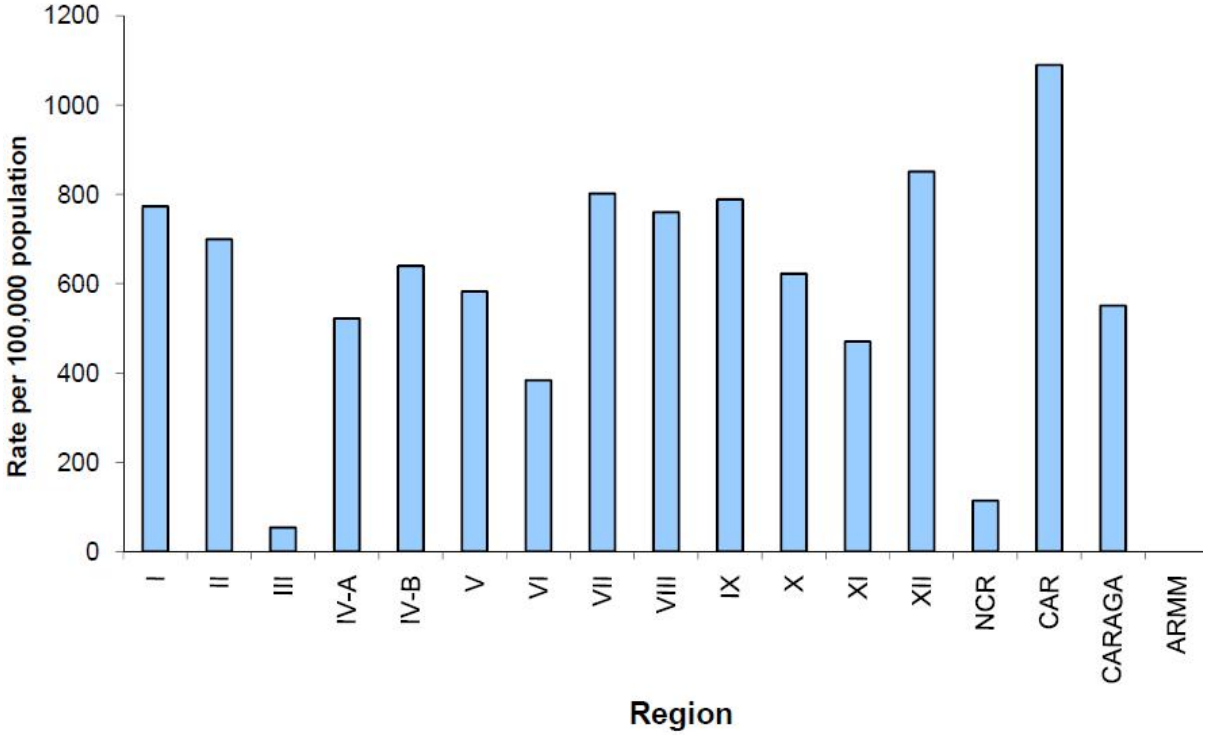


Figure 1. Acute watery diarrhea morbidity rate by year, Philippines 1995-2008

Source: FHSIS 2008



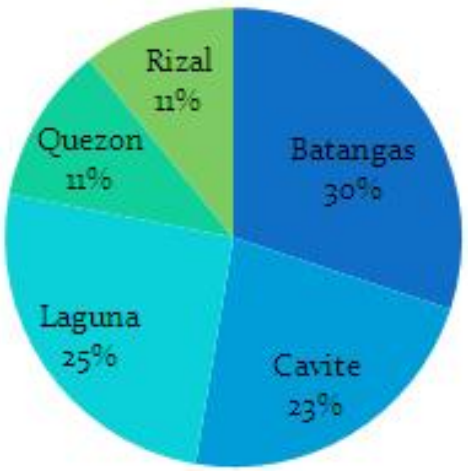
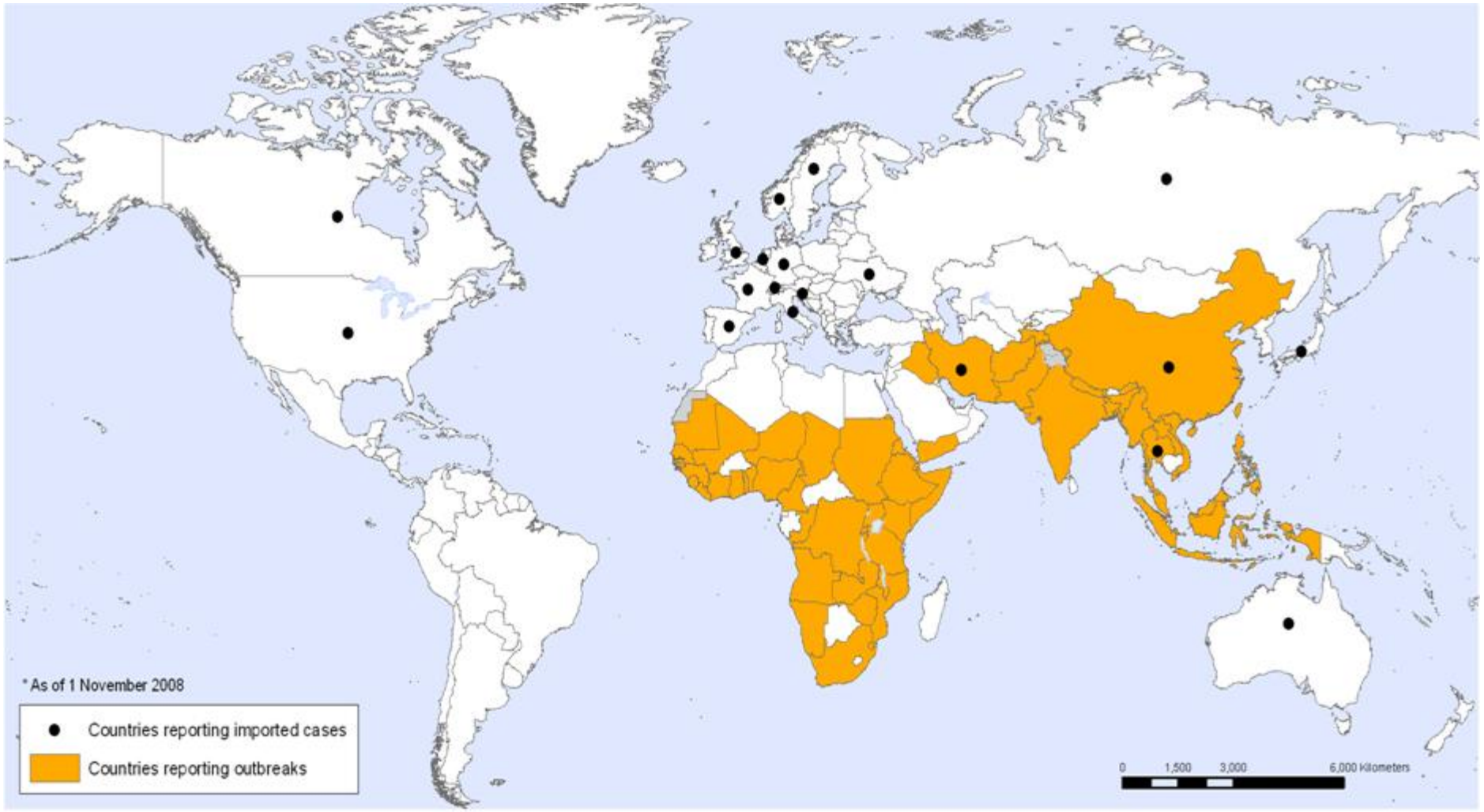


Figure 2. Acute watery diarrhea morbidity rate by region (bar graph) and by province (circle graph), Philippines 2008

Source: FHSIS 2008

Figure 3. Shellfish beds in Bacoor bay, Cavite

Figure 4. Situation of the coastal areas in Bacoor bay, Cavite



The boundaries and names shown and the designations used on this map do not imply the expression of any opinion whatsoever on the part of the World Health Organization concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. Dotted lines on maps represent approximate border lines for which there may not yet be full agreement.

Data Source: World Health Organization
Map Production: Public Health Information and Geographic Information Systems (GIS)
World Health Organization

World Health Organization

Figure 5. Countries reporting outbreak and imported cases of cholera

Source: WHO 2008

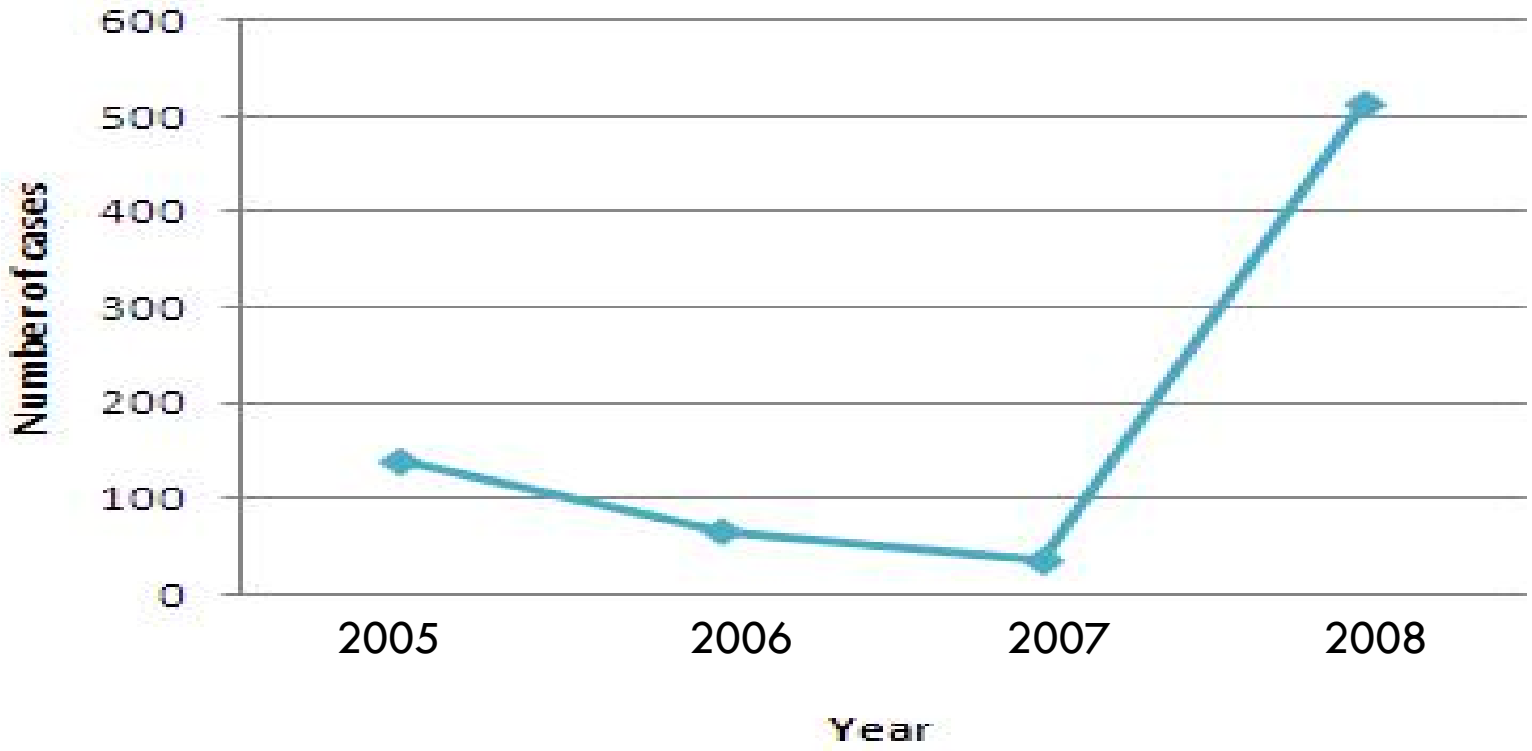


Figure 6. Cholera morbidity rate by year, Philippines 2005-2008

Source: PIDSR 2008

- *Vibrio* is considered highly susceptible to virtually all antimicrobials (Oliver 2006).
- Antimicrobial resistance has emerged and evolved in many bacterial genera due to the excessive use of antimicrobials in human and agricultural systems (Cabello 2006).
- In the Philippines, strains with transferrable, multiple drug resistance genes were isolated (Kobari *et al*. 1970; Kuwahara *et al*. 1967).
- Tetracycline resistance of *V. cholerae* has emerged wherein the genes are carried by transmissible plasmids (Brooks *et al*. 2007).

Resistance of pathogenic vibrios

- Ampicillin, chlortetracycline, erythromycin (Vaseeharan *et al*. 2005).
- Lincomycin (Ottaviani *et al*. 2001)
- Cefotaxime, tetracycline and chloramphenicol (Zanetti *et al*. 2001; Sack *et al*. 2001)
- Amoxicillin, ampicillin, carbenicillin, cefuroxime, rifampicin and streptomycin (Majushi *et al*. 2005)

Objectives of the Study

1. To determine the occurrence of total vibrios in green mussel, *Perna viridis* in relation to sampling period, harvest site, conductivity, water temperature, salinity, and pH.
2. To determine the level of resistance of the isolated pathogenic vibrios on selected antimicrobial agents.

Methodology

- Sampling (harvest) sites
- Water analysis (Temperature, pH, Conductivity, Salinity)
- Sample collection and enrichment
- Determination of total vibrios
- Isolation of *Vibrio* spp.
- Screening and confirmation of pathogenic vibrios
- Antibiotic susceptibility of enteropathogenic vibrios
- Statistical analyses

Results

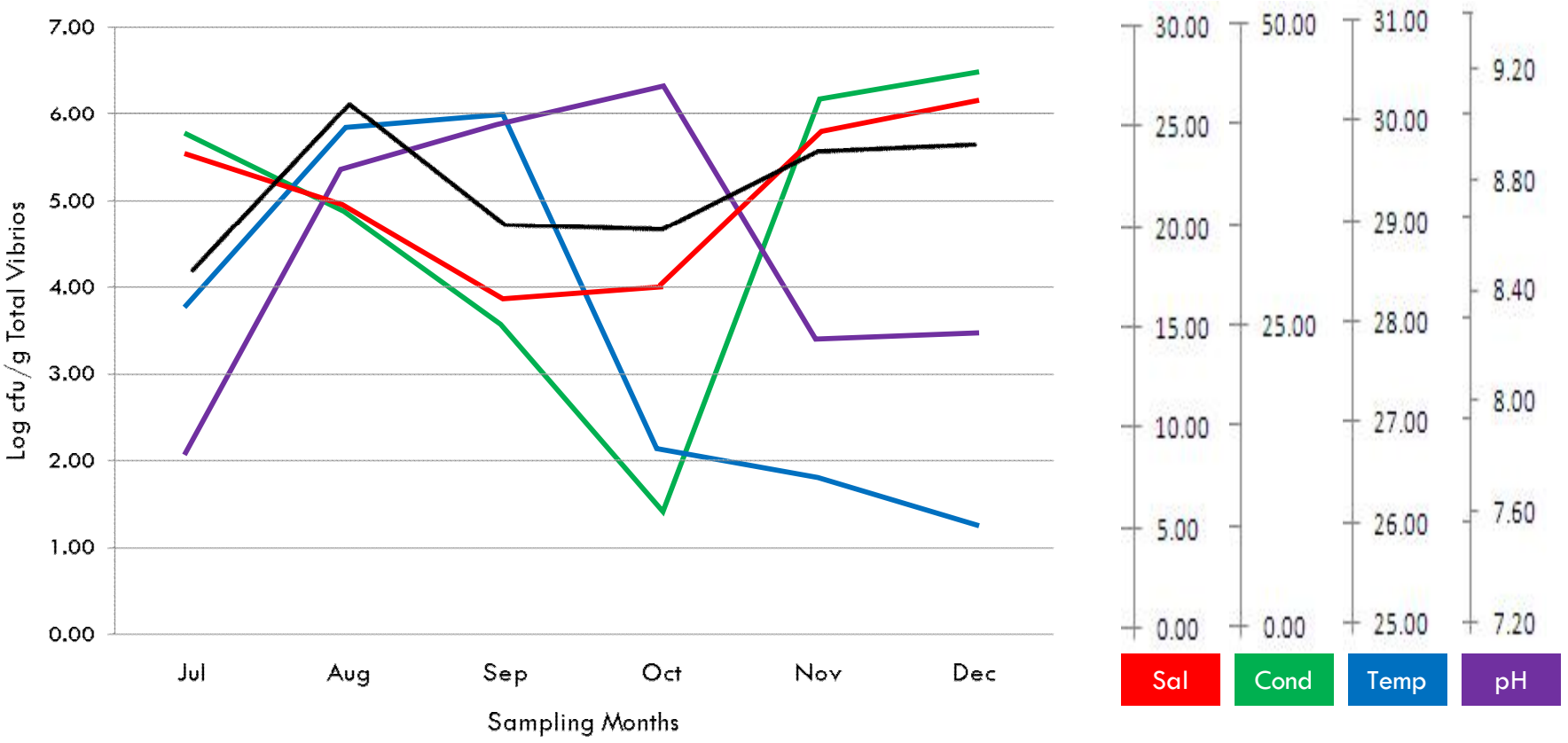


Figure 7. Average density of the total vibrios in relation to sampling months, salinity, pH conductivity, and water temperature

Table 1. Multiple correlation between the total vibrios and predictor variables.

Column Mean	Row Mean					
	Total Vibrios	Salinity	TDS	Conductivity	pH	Temperature
Total Vibrios	1.0000					
Salinity	0.1989	1.0000				
	0.4289					
TDS	-0.0290	0.5373	1.0000			
	0.9089	0.0215				
Conductivity	0.2162	0.9103	0.1576	1.0000		
	0.3889	0.0000	0.5322			
pH	0.1023	-0.8096	-0.356	-0.8043	1.0000	
	0.6862	0.0000	0.1472	0.0001		
Temperature	0.2204	-0.4819	-0.808	-0.1628	0.2782	1.0000
	0.3797	0.0428	0.0000	0.5185	0.2637	

Ho: There is no significant correlation between the total vibrios and predictor variables.

Table 2. Multiple linear regression model built using a stepwise backward elimination procedure.

Full model
p = 0.8428 Ó0.0500 removing TDS
p = 0.3249 Ó0.0500 removing Conductivity

Source	SS	df	MS			
Model	2.4×10^{12}	3	8.0×10^{11}	F (3, 14)	=	4
Residual	2.8×10^{12}	14	2.0×10^{11}	*p* > F	=	0.0299
Total	5.2×10^{12}	17	3.1×10^{11}	R^2	=	0.4617
				Adj. R^2	=	0.3463
				Root MSE	=	4.5×10^5

Total Vibrios	Coefficient	Std. Error	t	*p* > /t/	95% confidence interval	
Salinity	148532	45414	3.27	0.006	51128	245935
Temperature	187401	78194	2.40	0.031	19691	355112
pH	1071936	389055	2.76	0.015	237496	1906376
Constant	-1.7×10^7	5244308	-3.31	0.005	-2.86×10^7	-6.1×10^6

Ho: None of the predictor variables are good predictors of the total vibrio counts.

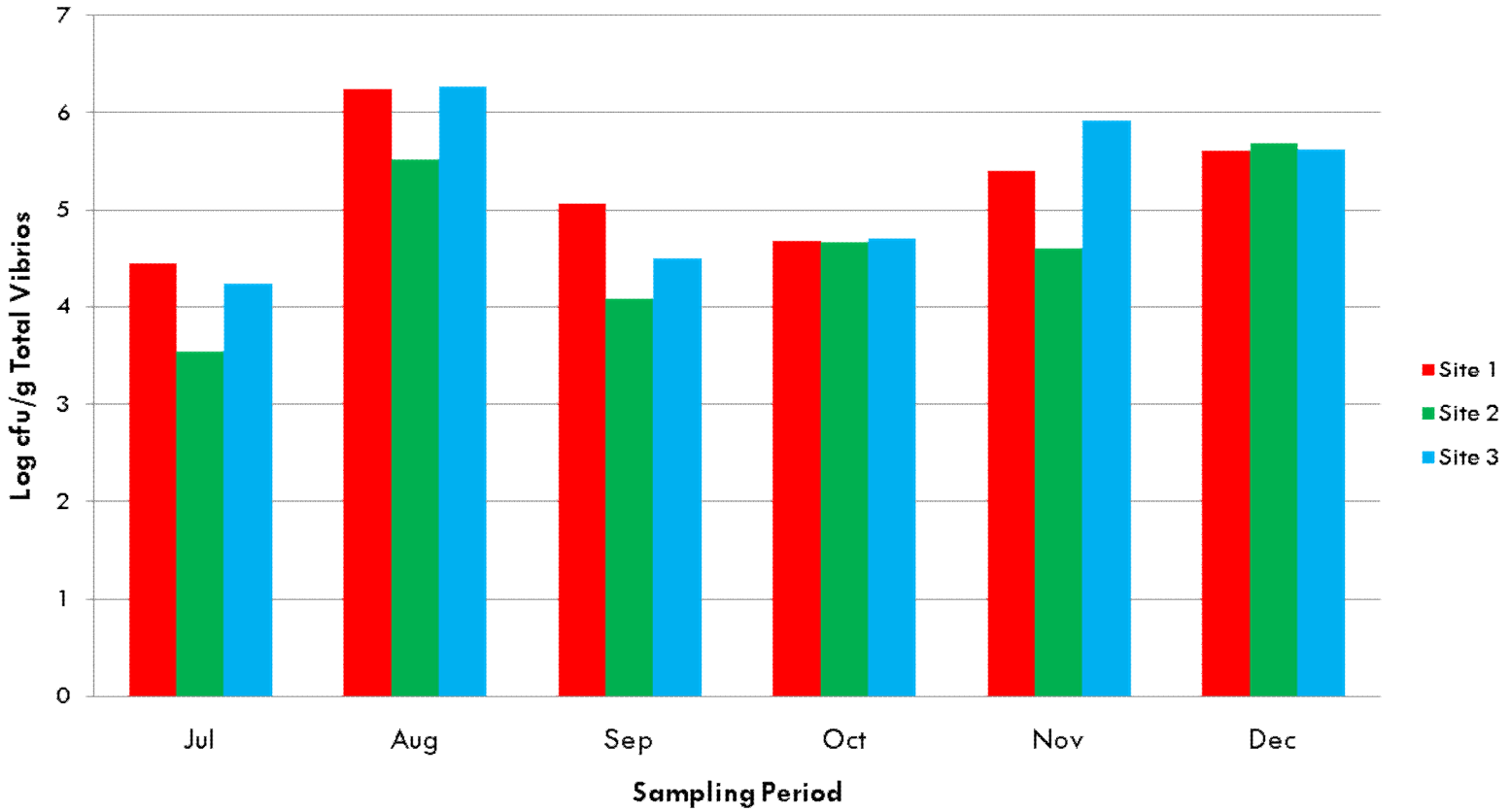


Figure 8. Average density of the total vibrios in relation to sampling months and harvest site

Figure 9. Sampling sites where mussels were collected.

Table 3. Biochemical reactions of the isolated strains of pathogenic vibrios.

Tests	Number of Positive Strains (%)			
	Vibrio alginolyticus N = 81	*Vibrio cholerae* N = 53	*Vibrio parahaemolyticus* N = 51	Other vibrios N = 85
Oxidase	81 (100%)	53 (100%)	51 (100%)	85 (100%)
Fermentation of sucrose	80 (99%)	53 (100%)	0(0%)	79 (93%)
Voges-Proskauer	80 (99%)	45 (85%)	0(0%)	0(0%)
Agglutination in poly O1 antiserum	ND	18 (34%)	ND	ND
Hemolysis	ND	ND	49 (96%)	ND
Growth in:				
0% NaCl	0(0%)	53 (100%)	0(0%)	0(0%)
3% NaCl	81 (100%)	53 (100%)	51 (100%)	85 (100%)
6% NaCl	78 (96%)	0(0%)	50 (98%)	15 (18%)
8% NaCl	77 (95%)	0(0%)	49 (96%)	0(0%)
10% NaCl	75 (93%)	0(0%)	0(0%)	0(0%)

ND - not determined

Table 4. Results of the biochemical tests of the twenty isolates in API® Identification Kit 20E (BioMerieux®).

Isolates	ONPG	ADH	LDC	ODC	CIT	H_2S	URE	TDA	IND	VP	GEL	GLU	MAN	INO	SOR	RHA	SAC	MEL	AMY	ARA	Identity
J1A1	-	-	+	+	+	-	-	-	+	-	+	+	+	-	-	-	+	-	-	-	VA
J1C2	+	-	+	+	+	-	-	-	+	-	+	+	+	-	-	-	+	-	-	-	VC
J3A1	-	-	+	+	-	-	-	-	+	-	+	+	+	-	-	-	+	-	-	-	VA
J3D3	+	-	+	+	-	-	-	-	+	-	+	+	+	-	-	-	+	-	-	-	VC
A3A2	-	-	+	+	-	-	-	-	+	-	+	+	+	-	-	-	+	-	-	-	VA
A3C2	-	-	+	+	-	-	-	-	+	-	+	+	+	-	-	-	-	-	-	-	VP
A3D1	-	-	+	+	+	-	-	-	+	-	+	+	+	-	-	-	+	-	-	-	VA
S1B3	+	-	+	+	-	-	-	-	+	-	+	+	+	-	-	-	+	-	-	-	VC
S1D2	+	-	+	+	+	-	-	-	+	-	+	+	+	-	-	-	+	-	-	-	VC
S2C3	-	-	+	+	-	-	-	-	+	-	+	+	+	-	-	-	+	-	-	-	VA
S3E3	-	-	+	+	-	-	-	-	+	-	+	+	+	-	-	-	+	-	-	-	VA
O1B1	+	-	+	+	-	-	-	-	+	-	+	+	+	-	-	-	+	-	-	-	VC
O2B3	-	-	+	+	-	-	-	-	+	-	+	+	+	-	-	-	-	-	-	-	VP
O2C3	+	-	+	+	+	-	-	-	+	-	+	+	+	-	-	-	+	-	-	-	VC
N1A1	+	-	+	+	+	-	-	-	+	-	+	+	+	-	-	-	+	-	-	-	VC
N1D1	+	-	+	+	-	-	-	-	+	-	+	+	+	-	-	-	+	-	-	-	VC
N1E3	-	-	+	+	-	-	-	-	+	-	+	+	+	-	-	-	+	-	-	-	VA
N2B2	+	-	+	+	-	-	-	-	+	-	+	+	+	-	-	-	-	-	+	-	VV
N2E2	-	-	+	+	+	-	-	-	+	-	+	+	+	-	-	-	+	-	-	-	VA
D3E1	+	-	+	+	-	-	-	-	+	-	+	+	+	-	-	-	-	-	+	-	VV

ONPG ï ß-galactosidase; ADH ï arginine dihydrolase; LDC ï lysine decarboxylase; ODC - ornithine decarboxylase; CIT ï citrate utilization; H_2S production; URE ï Urease; TDA ï tryptophan deaminase; IND ï indole production; VP ï acetoin production; GEL ï gelatinase; GLU ï glucose; MAN ï mannose; INO ï inositol; SOR ï sorbitol; RHA ï rhamnose; SAC ï saccharose; MEL ï melibiose; AMY ï amygdaline; ARA - arabinose
VA ï *V. alginolyticus*; VC ï *V. cholera*; VP ï *V. parahaemolyticus*; VV ï *V. vulnificus*

Table 5. Antibiotic susceptibility patterns of the isolated pathogenic vibrios.

Antibiotic tested (µg)	Number of resistant strains (%)					Number of sensitive strains (%)				
	VA	VC	VP	VV	Total	VA	VC	VP	VV	Total
Ampicillin (10 µg)	44 54%	31 58%	27 60%	0 0%	102 37.8%	28 35%	18 34%	15 33%	6 100%	67 24.8%
Chloramphenicol (30 µg)	3 4%	3 6%	2 4%	0 0%	8 3.0%	63 78%	38 72%	35 78%	6 100%	142 52.6%
Ciprofloxacin (5 µg)	3 4%	1 2%	0 0%	0 0%	4 1.5%	76 94%	49 92%	44 98%	6 100%	175 64.8%
Co-trimoxazole (25 µg)	9 11%	10 19%	6 13%	0 0%	25 9.3%	66 81%	38 72%	38 84%	6 100%	148 54.8%
Gentamicin (10 µg)	2 2%	0 0%	0 0%	0 0%	2 0.7%	79 98%	53 100%	45 100%	6 100%	183 67.8%
Neomycin (30 µg)	0 0%	0 0%	1 2.2%	0 0%	1 0.37%	66 81%	48 91%	38 84%	6 100%	158 58.5%
Nalidixic acid (30 µg)	12 15%	11 21%	5 11%	0 0%	28 10.4%	45 56%	26 49%	34 76%	6 100%	111 41.1%
Norfloxacin (10 µg)	0 0%	0 0%	0 0%	0 0%	0 0.0%	75 93%	50 94%	45 100%	6 100%	176 65.2%
Streptomycin (10 µg)	2 2%	1 2%	2 4%	0 0%	5 1.9%	62 77%	43 81%	38 84%	6 100%	149 55.2%
Tetracyline (30 µg)	10 12%	11 21%	7 16%	0 0%	28 10.4%	61 75%	37 70%	37 82%	6 100%	141 52.2%

VA – *V. alginolyticus*; VC – *V. cholerae*; VP – *V. parahaemolyticus*; VV – *V. vulnificus*

Table 6. Antimicrobial resistance profile of pathogenic vibrios isolated from mussels in Bacoor Bay, Cavite.

Antimicrobial resistance profile					Number of strains showing profile			
					VA	VC	VP	VV
NE	NA	TS	T	S	0	0	1	0
AP	NA	TS	T	C	0	1	0	0
AP	NA	TS	CIP	S	1	0	0	0
AP	NA	TS	C		1	1	0	0
AP	NA	T	C		1	2	0	0
AP	NA	T			4	2	1	0
AP	TS	T			1	2	0	0

VA ï *V. alginolyticus*; VC ï *V. cholerae*; VP ï *V. parahaemolyticus*; VV ï *V. vulnificus*
NE ï neomycin; AP ï ampicillin; NA ï nalidixic acid; TS ï co-trimoxazole; T ï tetracycline; C ï chloramphenicol; CIP ï ciprofloxacin; S ï streptomycin

- Resistant strains may have found their way in bays and harbored to these shellfishes because sewage and human wastes are discharged into these bodies of water.
- The isolation of pathogenic vibrios from mussels is a high risk for people consuming raw shellfishes.

Summary and Conclusion

- Vibrios were isolated from 90 mussel samples in shellfish beds of Bacoor bay, Cavite.
- The total vibrios were significantly correlated to sampling months ($p = 0.0114$) but not harvest sites and environmental parameters ($p > 0.05$).
- Salinity, water temperature and pH as predictor variables have significant effect on the density of total vibrios ($p<0.0309$).
- Of the 270 vibrio isolates
  - 79 strains were *V. alginolyticus*
  - 53 strains belonged to *V. cholerae* and 18 (34%) are *V. cholerae* serotype O1.
  - 49 strains belonged to *V. parahaemolyticus*
  - 7% of the remaining vibrios conformed to the characteristics of *V. vulnificus* based on the reaction in 8% NaCl and fermentation of sucrose.

Summary and Conclusion

- In antimicrobial sensitivity pattern, ampicillin had the highest drug resistance (37.8%) followed by nalidixic acid (10.4%), tetracycline (10.4%) and co-trimoxazole (9.3%).
- However, 8 strains of *V. alginolyticus*, 8 strains of *V. cholerae* with two being *V. cholerae* O1 and 2 strains of *V. parahaemolyticus* have at least triple resistance to selected antibiotics.
- The coastal area of Bacoor, Cavite is considered to be a significant reservoir of drug resistant vibrios.

Recommendation

Further Research

1. Determine the incidence, virulence factors and conjugation pattern of clinical and environmental pathogenic vibrios in coastal areas of Cavite.
2. Monitor the prevalence and antibiotic susceptibility profile of *Vibrio cholerae* and *Vibrio parahaemolyticus* during summer to better ensure the safety of oysters and mussels.

Local Government Units

1. Relocation of the people living in the bay area to break the transmission of vibriosis and cholera
2. Proper and thorough cooking of shellfishes harvested in the coastal areas of Bacoor, Cavite is a must.

ACKNOWLEDGEMENT

- University Faculty Research Office, DLSU-D

Thank you very much!