

DETECTION OF Salmonella Typhi in FARMED SHELLFISHES USING **CONVENTIONAL CULTURE METHOD AND POLYMERASE CHAIN REACTION, AND THEIR ANTIBIOTIC RESISTANCE**

An Undergraduate Research Presented to the Faculty of Biological Sciences Department College of Science and Computer Studies De La Salle University- Dasmariñas

Dasmariñas City, Cavite

In Partial Fulfillment of the Requirements

for the Degree of Bachelor of Science Major in Human Biology

MARIA KLENNA ROSE A. ISTINO

ANNA MARIE JOSIEFINE C. KAPULONG

March 2013

ABSTRACT

A study was performed in order to determine the prevalence of Salmonella Typhi and relate its presence in the type of farmed shellfishes (mussels and oysters) and season (hot dry, wet, cool dry) in Cavite City. A total of 30 samples (15 from mussels and 15 from shellfish) were collected from Cavite City during April, June and November. The physico chemical characteristics of water such as salinity, conductivity, temperature, dissolved oxygen and pH were also obtained. When the conventional culture method was performed, a 100% prevalence of Salmonella spp. was obtained from all the samples collected in different seasons. Using the PCR method, (ompC and viaB), a 90% prevalence of Salmonella spp. was detected in mussel isolates during the hot dry and wet season but not in dry season. On the other hand, Salmonella spp. isolated from oysters were prevalent during the hot dry and cool dry season and 70% prevalent in wet season. However, no Salmonella Typhi was observed in all of the samples. In determining the association between Salmonella spp. and predictor variables, the type of shellfish (P = 0.04) and season (P = 0.05) were found to be good predictors. Oysters obtained during hot dry season yield a favorable growth of Salmonella spp .In the antimicrobial susceptibility testing, 37% of bacterial isolates from the shellfishes were considered as multidrug resistant bacteria since it has shown resistance to the all of the antibiotics (ampicillin, chloramphenicol, streptomycin, and tetracycline).

TABLE OF CONTENTS

Title Page	1
Approval Sheet	2
Acknowledgments	3
Abstract	4
Table of Contents	5
List of Figures	7
List of Tables	8
CHAPTER 1 INTRODUCTION	10
1.1 Background of the Study	10
1.2 Conceptual Framework	13
1.3 Statement of the Problem	14
1.4 Scope and Limitations	15
1.5 Significance of the Study	15
1.6 Definition of Terms	17
CHAPTER 2 REVIEW OF RELATED LITERATURE	18
CHAPTER 3 METHODOLOGY	30
3.1 Research Design	30
3.2 Research Setting	30
3.3 Research Procedure	30
3.4 Data Gathering and Statistical Analysis	34

CHAPTER 5 CONCLUSIONS AND RECOMMENDATIONS 44 Cited References 46 Appendices 53 A. Photo documentation 54 B. Map of Study Site 60 C. Standard Procedure 61 D. Raw Data/Statistical Analysis 79 E. Curriculum Vitae 86	CHAPTER 4 RESULTS AND DISCUSSION	35
Cited References46Appendices53A. Photo documentation54B. Map of Study Site60C. Standard Procedure61D. Raw Data/Statistical Analysis79E. Curriculum Vitae86	CHAPTER 5 CONCLUSIONS AND RECOMMENDATIONS	44
Appendices53A. Photo documentation54B. Map of Study Site60C. Standard Procedure61D. Raw Data/Statistical Analysis79E. Curriculum Vitae86	Cited References	46
A. Photo documentation54B. Map of Study Site60C. Standard Procedure61D. Raw Data/Statistical Analysis79E. Curriculum Vitae86	Appendices	53
 B. Map of Study Site C. Standard Procedure D. Raw Data/Statistical Analysis F. Curriculum Vitae 	A. Photo documentation	54
C. Standard Procedure 61 D. Raw Data/Statistical Analysis 79 E. Curriculum Vitae 86	B. Map of Study Site	60
D. Raw Data/Statistical Analysis 79 E. Curriculum Vitae 86	C. Standard Procedure	61
6. Curriculum Vitae	D. Raw Data/Statistical Analysis	79
	E. Curriculum Vitae	86

De La Salle University - Dasmariñas isology program



Figure
1 - Shellfish samples washed in running water53
2 - Twenty-Four hour broth culture
3- Streak plate method55
4- Culturable Salmonella spp. growth detected in SSA55
5 - Nuclei Lysis solution was added
6 - PCR mastermix was prepared in microcentrifuge tubes
7 - Mixture was placed into the loading well
8- Gel stained with Ethidium Bromide
9 - Antimicrobial disks in Mueller Hinton Agar
10 - Site maps: Dasmariñas City, Cavite City and Bacoor City60

Table

1- Prevalence of the total Salmonella spp and Salmonella Typhi isolated in
farmed shellfishes from April, June and November 201235
2-Physical characteristics of sampling site from April to November
2012
3-Antimicrobial Resistance Patterns of Salmonella spp. from farmed
shellfishes
4- Multidrug Resistance of the Isolates (Salminella spp.) in mussels and
oyster40
5- Presence of culturable <i>Salmonella spp.</i> in April Samples
6- Presence of culturable <i>Salmonella spp</i> . in June Samples78
7- Presence of culturable <i>Salmonella spp</i> . in November Samples79
8- Presence of <i>ompC</i> gene and <i>viaB</i> gene in April Isolates79
9- Presence of <i>ompC</i> gene and <i>viaB</i> gene in June Isolates
10 - Presence of <i>ompC</i> gene and <i>viaB</i> gene in November Isolates80
11- Physical Parameters of Sampling Site for April 201281

De La Salle University - Dasmariñas isology program