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Occurence of Pathogens from Major Shrimp and Oyster Production Areas in Peninsular Malaysia

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Abstract. - This paper attempts to report on the possible isolation of human pathogens especially *Salmonella* sp., *Vibrio cholera* and *Vibrio parahaemolyticus* in shrimps (*Penaeus monodon, Penaeus merguiensis*) and oysters (*Crassostrea irradelei)* cultured in Peninsular Malaysia. The presence of pathogens was determined using standard microbiological methods. *Vibrio cholera* and *V. parahaemolyticus* were not isolated from all of the shrimp and oyster samples tested. A total of 102 *Salmonella* were isolated but only 69 were able to be serotyped with 6 Kauffmann-White groups encountered. *Salmonella weltevreden* was the most common isolate and to the lesser extent *S. hvittingfoss*, *S. Litchfield*, *S. Newport*, *S. agona*, *S. paratyphi B*, *S. edinburg*, *S. benin*, *S. java*, and *S. oslo*. All of the *Salmonella* serotypes isolated from the shrimp and shrimp culture environment were resistant to erythromycin. *Salmonella hvittingfoss* showed multiple resistance to choramphenicol, erythromycin, furazolidone, streptomycin and tetracycline. *Salmonella* serotypes isolated from oysters were sensitive to all the antibiotics tested. Processors and consumers should be concerned with the presence of *Salmonella*, including pathogenic serotypes, and apply adequate measures to ensure the safety of the products.

Keywords: Pathogens, shrimp, oysters, Peninsular Malaysia

Abstrak. - Kertas ini melaporkan tentang percubaan untuk memencilkan patogen manusia terutamanya *Salmonella* sp., *Vibrio cholera* dan *Vibrio parahaemolyticus* dalam udang (*Penaeus monodon, Penaeus merguiensis)* dan tiram (*Crassostrea irradelei)* yang diternak di Semenanjung Malaysia. Kehadiran patogen dikesan dengan kaedah piawai mikrobiologi. Keputusan menunjukkan *V. cholera* dan *V. parahaemolyticus* tidak dikesan dalam semua sampel udang dan tiram yang diperiksa. Sejumlah 102 *Salmonella* telah berjaya dipencilkan tetapi cuma 69 isolat disahkan sebagai serotip kepada 6 Kumpulan Kaufmann-White. *Salmonella weltevreden* dikenalpasti sebagai isolat *Salmonella* yang paling kerap dipencilkan diikuti dengan *S. hvittingfoss*, *S. Litchfield*, *S. Newport*, *S. agona*, *S. paratyphi B*, *S. edinburg*, *S. benin*, *S. java* dan *S. oslo*. Kesemua serotip yang dipencilkan dari udang dan kawasan ternakan udang menunjukkan kerintangan pada antibiotik erythromycin. *Salmonella hvittingfoss* pula menunjukkan ciri-ciri kerintangan berganda kepada chloramphemicol, erythromycin, furazolidone, streptomycin dan tetracycline. *Salmonella* yang dipencilkan dari tiram pula adalah sensitif kepada semua antibiotik yang diuji. Kehadiran *Salmonella* terutamanya serotip yang patogenik sepatutnya di ambil perhatian oleh pemproses dan pengguna supaya langkah-langkah yang bersesuaian dapat diambil untuk memastikan keselamatan dalam menggunakan produk ini.

Introduction

Food-borne diseases caused by human pathogens such as *Salmonella, Vibrio cholerae* and *Vibrio parahaemolyticus* pose a serious public health problem in many parts of the world. Data from food-borne disease surveillance programmes in developed countries indicate that *Salmonella* is the leading cause of food-borne infections (CAST Report, 1994). In the United States for example, approximately 25,000 *Salmonella* infections were reported annually in the 1970's. There had been a continual increase to 41,222 reported infections in 1995 (Thomas *et al.*, 1997). Besides *Salmonella*, *V. cholerae* and *V. parahaemolyticus* are also major pathogens

causing food poisoning. It was reported that incidents of *V. parahaemolyticus* food poisoning in Japan were the second most prevalent after that of *Salmonella* (The Fish Inspector, 1999).

Salmonella are distributed all over the world, but naturally occurs in the gut of animals. They are not naturally present in seawater. *Salmonella* are gram negative, mobile bacilli that can grow both aerobically and anaerobically between 7°C and 48°C (optimum 37°C) at pH 4.0 - 8. They are readily killed by heat (>70°C for 75 seconds) and acid (1.4% acetic acid and at pH 4.0 within 72 hours). They are also resistant to both freezing and drying particularly in the presence of proteins and other protectants (D' Agust *et al.*, 1989). On the other hand, *V. cholerae* and *V. parahaemolyticus* are among the most common organisms in surface waters of the world. They occur in both marine and freshwater habitats and in association with aquatic animals.

Food infections caused by *Salmonella* have been associated with raw oysters, salmon, tuna salad, shrimp cocktail, stuffed sole and fish fillet. Most human *Salmonellosis* manifest as a gastroenteritis with the infection confined to the gastrointestinal tract. Meanwhile, *V. parahaemolyticus* and *V. cholerae* are usually associated with consumption of contaminated shellfish, especially oyster, and both cause diarrhea but in ways that are entirely different. In addition to infection, the emergence of antibiotic resistant bacteria further complicates the problem. Antibiotics are widely used for disease control in both man and animals. Food animals are now the major source of antimicrobial resistant infection in man with the use of antimicrobial drugs in farms (Technical Report, 1988).

Shrimps and oysters are among the popular seafood in Malaysia and also globally. At present, shrimps are the most important aquaculture product in terms of generating high income to the farmers and fisheries industry. One of the problems faced by the shrimp industry, especially in the tropics, is the implementation of stringent microbiological standards set by importing countries. The United States Food and Drug Administration (USFDA) had issued 3,904 "Notice of Detention and Hearing" for seafood products during the period of January to October 1999, averaging slightly more than 390 detention notices per month (U.S. Seafood News, Vol. 7 (12), Nov. 1999). Most of the detentions were from countries in Asia and the shrimps were suspected of having *Salmonella* or filth. The detectable presence of *Salmonella* is considered as indicator of adulterated seafood by the importing countries (Ahmed, 1991). However, it was argued that these standards were only applicable to marine products, since the microbial population of tropical aquaculture product is somewhat different. This argument is strengthened by reports and findings of Reilly and Twiddy (1992), who claimed that *Salmonella* might be a natural population of the brackish water cultured shrimp rather than as a result of a poor standard in hygiene and sanitation during processing. However it cannot be denied that the presence of pathogenic bacteria in oyster is a much bigger risk since it is generally consumed raw or half cooked. Stringent microbiological standards in oyster should be applied in this case.

The presence of human pathogenic bacteria in our seafood and seafood products will give a negative impact to the fisheries industry. Other possible consequences are reduction in the value of the products, rejection of consignments, legal actions or in worse cases, outbreaks of food poisoning. Based on this concern, investigations were carried out to find out and evaluate the extent of human pathogen occurrences in the shrimp and oyster culture areas. The antibiotic susceptibility of the isolates was also determined to evaluate the extent of

resistance. Results from this study will be used to guide farmers and regulatory agencies to undertake suitable follow-up actions to ensure that these product are safe for human consumption.

Materials and Methods

Collection of samples

The types and number of samples analysed during the study period are shown in Table 1. A total of 242 oyster samples were collected once a fortnight from February until mid December 1998. Samples were collected aseptically using a sterile bottle and bag at each sampling station and appropriately labelled. All the samples were brought to the laboratory in an ice-cooled insulated box and due care was given to ensure that the samples did not come in direct contact with the ice in the box. The samples were immediately analysed upon reaching the laboratory.

Table 1 : Types and number of samples from shrimp cultured environment analysed during the study period (February - December 1998) in the various states

States	Types Samples			
	Sediment	Pond Water	Shrimp	Formulated Feed
		No of samples		
Perlis	5	30	30	5
Kedah	10	55	70	5
Penang	5	30	30	5
Perak	10	40	70	5
Selangor	10	45	45	5
Terengganu	-	45	60	-
Johor	-	35	35	-

Pathogen isolation and identification

Sample preparation and pathogen isolation were carried out according to the methods described by APHA (Compendium of Methods for the Microbiological Examination of Foods, 3rd Edition, (1992) and Standard Methods for the Examination of Water and Wastewater, 17th Edition, (1989)). The confirmation of pathogen was carried out using the bacteria identification kit API 20 E System (BioMérieux sa) and the interpretation was performed using computer and cross-checked with API 20 E Analytical Profile Index (3rd Edition). Cultures of *V. cholerae* (V63), *V. parahaemolyticus* (V104) and *Salmonella typhi* (S85) obtained from the Institute for Medical Research, Kuala Lumpur were used as reference materials in the isolation of these pathogens.

Serotyping

Suspected *Salmonella* spp. isolates were characterized serologically by specific antigenic components. The method adopted for *Salmonella* serotyping was slide agglutination using SA Scientific Incorporation 'O' and 'H' antisera. Strains were grouped into serotypes based on their reactions to group and specific antisera according to the modified Kauffmann-White Scheme.

Antimicrobial susceptibility test

Confirmed isolates were screened for resistance to Streptomycin (S-10ìg), Tetracycline (T-30ìg), Erythromycin (E-15ìg), Chloramphenicol (C-30ìg) and Furazolidone (F-100ìg). *Salmonella* isolates were suspended in saline to the density of a Mac_Farland No. 2 Standard, and streaked by the method of Bauer *et al.* (1966) on Mueller Hinton Agar. Plates were incubated for 24 hours at 37°C. Characterization of strains as sensitive, intermediate or resistant was based on the size of inhibition zones around each antobiotic tested according to the manufacturer's instructions (BBL Sensidisc, Becton Dickinson, USA). A control culture of *E. coli* (K12-Nalidixic acid resistant) were obtained from University of Malaya, Kuala Lumpur.

Results and Discussion

The predominant microflora found in shrimps and oysters comprise Pseudomonads (*Pseudomonas putida*, *Pseudomonas* spp.), Enterics (*Escherichia coli*, *Edwarsiella tarda*, *Citrobacter freundii*, *Enterobacter agglomerans, Enterobacter cloacae*, *Hafnia alvei, Serratia liquefaciens, Serratia marcescens*, *Proteus mirabilis, Klebsiella pneumoniae* and *Erwina* sp.), yellow orange pigmented bacteria (*Flavobacterium branchiphilum*, *Flavobacterium odoratum*, *Flexibacter* spp. and Myxococcus), Vibrios (*Vibrio harveyi*, *Vibrio fischeri*) and Aeromonads (*Aeromonas sobria*, *Aeromonas* spp.) .

A total of 102 *Salmonella* were isolated but only 69 *Salmonella* isolates were serotyped and 6 serotypes encountered. The most frequent serotype encountered over the period of study are listed in Table 2. Isolates were mainly from Kauffman-White Groups namely E1, I(016), C1, C2, B and D2. The frequency of isolation is indicated in Table 3. *Salmonella weltevreden* accounted for nearly half of the total number of *Salmonella* isolated . Of these 69 isolates 40.6% were *S. weltevreden*, 17.4% were *S. hvittingfoss*, 10.1% were *S. newport*, 8.6% were *S. Litchfield*, 5.8% were *S. agona*, 4.3% were *S. paratyphi B*, 4.3% were *S. benin*, 4.3% were *S. edinburg*, 2.9% were *S. java* and to a lesser extent *S. oslo* (1.4%). From the results obtained there is a marked difference in the serotype found in shrimp and shrimp culture environment and to that found in oysters. *Salmonella weltevreden* are the major isolates in shrimp and shrimp culture environment while *S. newport* is the major isolate from oysters. *Salmonella hvittingfoss* was the only serotype found in both shrimp and oyster samples in this study.

Table 2 : *Salmonella* species isolated during study period February-December 1998

***Salmonella* type**	**Group**	**Somatic (0) Antigen**	**Flagellar (H) Antigen**	
			Phase 1	**Phase 2**
S. weltevreden	E1	3, 10	r	$^{z}6$
S. hvittingfoss	1	16	b	e, n, x
S. newport	C2	6, 8	e, h	1, 2
S. litchfield	C2	6, 8	l, v	1, 2
S. agona	B	1, 4, 12	f, g, s	-
S. edinnburgh	C1	6, 7	b	1, 5
S. oslo	C1	6, 7	a	e, n, x
S. paratyphi B	B	1, 4, [5], 12	b	1, 2
S. java	B	1, 4, [5], 12	b	[1, 2]
S. benin	D2	9, 4	y	1, 7

Table 3 : Frequency of *Salmonella* isolated from February-December 1998

Serotypes	Number of isolation
From shrimp and shrimp culture environment	
S. weltevreden	28
S. hvittingfoss	11
S. litchfield	6
S. agona	4
S. paratyphi B	3
S. benin	3
S. java	2
From oyster	
S. newport	7
S. edinburg	3
S. oslo	1
S. hvittingfoss	1

Distribution of *Salmonella* in shrimps and shrimp culture environments in the various states of Peninsular Malaysia is illustrated in Fig. 1. *Salmonella weltevreden* showed a wider distribution and was present in samples from Perlis, Kedah, Penang, Perak and Selangor. Detections were mostly on samples obtained from Perak and Selangor especially in traditional farms as well as those which are located near poultry farms or integrated farms. *Salmonella* was not detected from samples examined in Johor and Terengganu in this study period. It was also observed that *Salmonella* in shrimp and shrimp culture environments can be detected all year round.

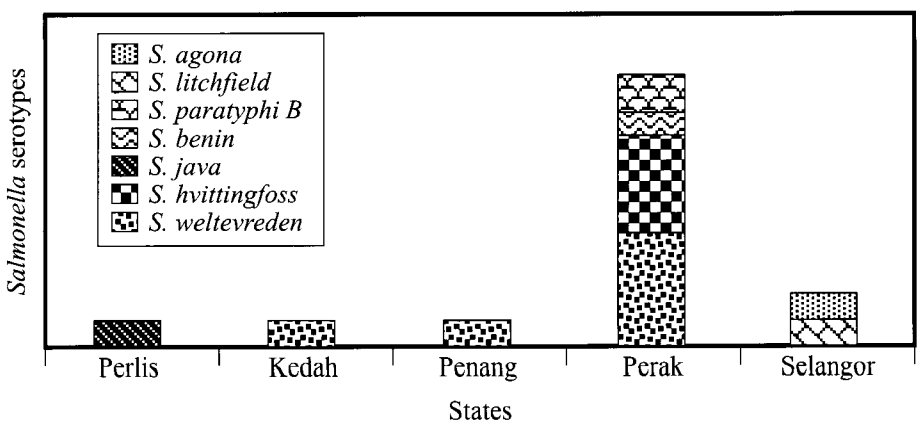

Figure 1 : Distribution of *Salmonella* isolates from shrimp and shrimp culture environment in Peninsular Malaysia

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