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OCCURRENCE OF *SALMONELLA* AND *CAMPYLOBACTER* SPP. IN BIVALVE MOLLUSCS RETAILED IN SELANGOR, MALAYSIA

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SUMMARY

The consumption of raw or insufficiently cooked shellfish which include bivalve molluscs can cause food-borne diseases, such as salmonellosis and campylobacteriosis. This study was carried out to determine the occurrence of *Salmonella* and *Campylobacter* in blood cockles (*Anadara granosa*) and carpet clams (*Paphia undulate*) retailed in the markets. Twenty samples each of blood cockles and carpet clams (total 40 samples) were purchased from markets in Selangor. Sixteen or 40% of the samples were found positive for *Salmonella* species. The most frequently isolated serotypes were *Salmonella* Corvalis (31.3%), followed by *Salmonella* Mikawasima and *Salmonella* Weltevreden (18.8% each), *Salmonella* Tennessee (12.5%), *Salmonella* Agona, *Salmonella* Pomona and *Salmonella* Typhimurium (6.3% each). *Campylobacter* was not isolated. This study shows the potential risk of acquiring salmonellosis from ingesting raw or undercooked blood cockles and carpet clams.

Keywords: *Campylobacter, Salmonella,* blood cockles, carpet clams, bivalve molluscs

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INTRODUCTION

Shellfish, in particular the bivalve molluscs, such as mussels, oysters, clams and cockles, are known to be carriers of bacterial and viral pathogens. This is because being filter feeders, they ingest and concentrate all particulate matters in the water including pathogenic organisms (Martinez-Urtaza *et al*., 2003). As such, these bivalve molluscs are considered a major food safety concern because when consumed raw or inadequately cooked, they have been reported to cause foodborne illnesses. Consumption of raw oysters and other shellfish has been linked to outbreaks of hepatitis A and viral gastroenteritis such as norovirus, rotavirus, enterovirus and astrovirus infections (Brands *et al*., 2005; Le Guyader *et al.,* 2000). Bacterial pathogens, reported to occur in shellfish and cause foodborne illnesses, include pathogenic *E. coli*, *Vibrio cholerae*, *Vibrio parahaemolyticus*, *Campylobacter jejuni, Listeria monocytogenes*, *Staphylococcus aureus* and *Salmonella* spp. (Brands *et al.,* 2005; Ripabelli *et al*., 1999). *Arcobacter*, considered as an emergent foodborne and waterborne pathogen, has been isolated from clams (5/5,100%) and mussels (23/56, 41.1%) with *Arcobacter butzleri* being the most prominent species; none were isolated from oysters and frozen shrimps (Collado *et al*., 2009).

In Malaysia, the shellfish industries contributed 44% to aquaculture production in 2001. The bivalve molluscs such as blood cockles (*Anadara granosa*), green mussels (*Perna viridis*) and carpet clams (*Paphia undulata*) are cultured mainly along the west coast of Peninsular Malaysia (Wan Norhana and Nor Ainy, 2004). The bivalves are popular among Malaysian consumers. They are usually 'steamed' and eaten with sauce or cooked in a variety of dishes. In many countries including Malaysia, the harvesting areas of the bivalves have become more populated in recent years with more human sewage being discharged into coastal waters. It has resulted in an increase in pathogens in these waters which serve as a growing environment for the molluscs (Martinez-Urtaza *et al.,* 2003). These in turn cause a higher incidence of foodborne diseases from such shellfish. According to Shabarinath *et al.* (2007), aquatic environments, which are major reservoirs of *Salmonella,* enhance their transmission between hosts. The survival rate of *Salmonella* in such aquatic environments is very high, outliving even *Vibrio cholera*. It is also reported that *Salmonella* in aquaculture products usually originates from the environment and is not caused by poor management and hygiene practices or using poultry litter as feed (Sanath Kumar *et al.,* 2003).

Shore birds such as seagulls are implicated as the primary source of *Campylobacter* contamination in shellfish (Jacobs-Reitsma *et al.,* 2003; Jones and Obiri-Danso, 1997). *Campylobacter* infections in humans are frequently associated with consumption of poultry meat and poultry products; other risk factors include drinking raw milk, untreated surface and contaminated drinking water and contact with infected pet animals. The risk

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posed by red meat is less but has increased together with raw fruits, vegetables and unpasteurised fruit juices (Humphrey *et al.,* 2007). Apart from shellfish, *Campylobacter* has also been isolated from 21% of crab meat from processing facilities (Jacobs-Reitsma, 2003).

The aim of this study is to determine the presence of *Salmonella* and *Campylobacter* in blood cockles (*Anadara granosa*) and carpet clams (*Paphia undulate*) retailed in markets in Selangor.

MATERIALS AND METHODS

Collection of samples

Forty (40) samples of bivalves, consisting of 20 samples each of blood cockles (*Anadara granosa*) and carpet calms (*Paphia undulate*) were purchased from markets around Klang Valley areas in Selangor, Malaysia. The samples (each weighing 100 g) were packed individually in a plastic bag, placed in an insulated box containing ice packs and transported to the laboratory. The bivalves in each sample were washed, dried, disinfected with 70% ethanol and opened aseptically using a sterilised knife and forceps. 10 g of the inner contents (flesh) and the internalised water (liquor) of the bivalves were then placed in 90 ml of appropriate enrichment broth and homogenised for 1 min.

Isolation of Salmonella

The contents of the bivalves were placed in Buffered Peptone Water (Oxoid). After homogenisation, they were incubated aerobically at 35°C for 24 h. One ml of the pre-enriched sample was transferred into 9 ml of Rappaport-Vassiliadis broth (Oxoid) and incubated at 42°C for 48 h under aerobic condition. After incubation, a loopful of the enriched sample was streaked onto two selective agar – (1) XLT4 agar base (BD Difco) with XLT4 supplement (BD Difco) added according to manufacturer's directions of use and (2) Rambach agar (Merck). All plates were incubated aerobically at 37°C for 24 h.

Identification and serotyping of Salmonella

Suspected colonies on agar plates were subcultured for purity and then subjected to biochemical tests, which included Triple Sugar Iron (TSI) agar (Oxoid), Lysine Iron Agar (LIA) (Oxoid), Sulfide Indole Motility (SIM) agar (Oxoid) and urease test. Slide agglutination test (SAT) was done on presumptive *Salmonella* isolates using *Salmonella* O Polyvalent Antisera, Poly. A-S (Serotest). The isolates positive to SAT were sent to the Veterinary Research Institute (VRI) for confirmation and serotyping of the *Salmonella* isolates using the Kaufmann White Group classification.

Isolation of Campylobacter

The contents of the bivalves were placed in an enrichment broth consisting of Brucella broth (BD Difco) with 5% laked horse blood (Oxoid), CCDA Medium Selective Supplement (Oxoid) and Campylobacter Growth Supplement (Oxoid) incorporated according to manufacturer's instructions. After homogenisation, the samples were incubated at 42°C for 48 h under microaerophilic conditions generated by using an anaerobic jar containing a gas generating pack (GasPak EZ Campy, BD). Following incubation, a loopful of each enriched sample was streaked onto Campylobacter blood-free selective agar base (Modified CCDA-Preston, Oxoid) with CCDA Medium Selective Supplement (Oxoid) added. All plates were incubated at 42°C for 48 h under microaerophilic conditions as mentioned.

Identification and confirmation of Campylobacter

Suspected colonies were picked for Gram staining, motility observation under wet mount and catalase test. Colonies giving reactions typical for *Campylobacter* were subcultured so as to obtain pure cultures. *Campylobacter* species were confirmed and speciated using MAST ID™ Camp Identification System (Mast Diagnostics) which consists of three biochemical tests, namely hippurate hydrolysis, indoxyl acetate hydrolysis and urease tests. This kit differentiates *Campylobacter* isolates into *Campylobacter jejuni, C. coli* and *C. lari*.

RESULTS

A summary of the results is shown in Table 1. Of the 40 samples examined, 16 (40%) were found positive for *Salmonella. Salmonella* was isolated from 7 (35%) of the blood cockles and 9 (45%) of the carpet clams. The most frequently isolated serotypes were *Salmonella* Corvalis (31.3%), followed by *Salmonella* Mikawasima and *Salmonella* Weltevreden, (18.8% each), *Salmonella* Tennessee (12.5%), *Salmonella* Agona, *Salmonella* Pomona and *Salmonella* Typhimurium (6.3% each). In this study all the samples were negative for *Campylobacter*.

DISCUSSION

The study showed a high occurrence of *Salmonella* in the blood cockles and carpet clams. The growing presence of *Salmonella* in the waters had most probably led to its occurrence in the bivalves. Among the factors that resulted in high faecal materials as well as pathogens such as salmonellae, vibrios, pathogenic *E. coli* and campylobacters in seawater include runoff of agriculture, residential and wildlife wastes into local rivers and

Table 1: Isolation and identification of *Salmonella* from blood cockles (*Anadara granosa*) and carpet calms (*Paphia undulate*)

Bivalve samples	No. of samples	No. (%) positive for *Salmonella*	*Salmonella* serotypes identified and %
Blood cockles	20	7 (35%)	*Salmonella* Corvalis (42.9%) *Salmonella* Tennessee (28.6%) *Salmonella* Mikawasima (14.3%) *Salmonella* Agona (14.3%)
Carpet clams	20	9 (45%)	*Salmonella* Weltevreden (33.3%) *Salmonella* Corvalis (22.2%) *Salmonella* Mikawasima (22.2%) *Salmonella* Pomona (11.1%) *Salmonella* Typhimurium (11.1%)
Total	40	16 (40%)	7 serotypes indentified

streams and eventually seawater. Discharge of industrial and municipal effluents may also introduce these pathogens into the aquatic environment (Shabarinath *et al.*, 2007; Brands *et al.*, 2005; Le Guyader *et al.*, 2000). The findings in this study were almost similar to that of Wan Norhana and Nor Ainy (2004) who reported the presence of *Salmonella* in cockles, clams, oysters and green mussels at 45.5%, 27.3%, 9.1% and 18.2%, respectively. Ten serotypes were identified in the study. Other studies, mostly on oysters and mussels, showed the presence of salmonellae ranging from 7.4% to 30% (Brands *et al.*, 2005; Shabarinath *et al.*, 2007). Several studies used a conventional method to detect the presence of *Salmonella.* Sanath Kumar *et al.* (2003) reported that if molecular techniques such as PCR were used, a higher occurrence rate could be detected. Shabarinath *et al.* (2007) similarly reported a higher recovery rate; they found only 7% of oysters and 33% of clams positive for *Salmonella* by using the culture method as compared to 30% of oysters and 50% of clams positive when using direct enrichment lysate PCR technique.

Brands *et al.* (2005) reported *S.* Newport and Shabarinath *et al.* (2007) reported *S.* Weltevreden as the major serotypes identified. WHO in 2005 reported *S.* Weltevreden as the important cause of non-typhoidal salmonellosis in South East Asia and Western Pacific compared to Western Europe and United States where it was seldom isolated (Shabarinath *et al.*, 2007). In Malaysia, *S.* Enteritidis, *S.* Agona, *S.* Weltevreden and *S.* Typhimurium were among the frequently isolated serotypes from animals and livestock products from 1996 to 2001 (Maria *et al.*, 2002). *S.* Weltevreden was the most frequently isolated serotype from indigenous vegetables, followed by *S.* Agona (Yoke-Kqueen *et al.*, 2008). From 1989 to 1992, *S.* Weltverden was the third leading serotype but since 1993, more than 30% of salmonellosis among Malaysians was due to *S.* Enteritidis (Yasin *et al.*, 1997). It has been reported that warm waters may allow increased bacterial survival of *Salmonella* and *E. coli* (Rhodes and Kator, 1988). There have been studies in which *Salmonellae* were not found in bivalves (Jacob-Reitsma *et al.*, 2003); Ripabelli *et al.*, 1999). It was observed by Brands *et al.* (2005) that the presence of *Salmonella* in oysters varies according to geographical areas, seasons and water related activities. The absence of *Campylobacter* in this study was similarly reported by Ripabelli *et al.* (1999) but a number of studies report high occurrence rates of campylobacters, ranging from 19% to 42% (Jacob-Reitsma *et al.*, 2003; Wilson and Moore, 1996). Wee *et al* (2007) too did not isolate *Campylobacter* from seafood (cockles, white shrimps, sardine fishes and squids) from the northern states of Malaysia but 50% of the freshwater bivalves (edible corbiculas) and apple snails were positive for *Campylobacter coli* using both the conventional method and multiplex PCR technique. With their findings of *Campylobacter lari* in mussels and oysters, Jones and Obri-Danso (1997) and Jacob-Reitsma *et al.* (2003) reported that campylobacters in seawater and bivalves came from faeces of wildbirds, such as seagulls which contaminate the aquatic environment, rather than from sewage effluents. Apart from *Salmonella*, Wan Norhana and Nor Ainy (2004) isolated vibrios, mainly from cockles (75%), *Vibrio parahaemolyticus* from cockles, clams and oysters and *V. cholerae* from cockles only. Ripabelli *et al* (1999) found that 48.4% of mussels contained vibrios with *V. alginolyticus* as the most frequently isolated species followed by *V. vulnificus*.

The presence of *Salmonella* in this study and other pathogens such as *Campylobacter* and *Vibrio* in cockles and clams as reported in other studies in Malaysia shows their widespread occurrence which is of public health concern. This is because such contaminated shellfish can cause foodborne illnesses when consumed raw or undercooked. Wan Norhana and Nor Ainy (2004) state that the bacteriological quality of bivalves is below the recommended guideline. Hence, treatment is suggested,

such as relaying combined with purification so as to reduce the microbial loads of the bivalve molluscs prior to sale in the markets. From their study, Ho and Tam (2000) observed that with a high accumulation of microorganisms in the mussels, natural depuration or purification might not be effective in achieving the acceptable microbiological quality for human consumption. According to Brands *et al.* (2005), monitoring should therefore be carried out to determine the suitability of shellfish for human consumption by testing bivalve flesh specifically for these pathogens on a regular basis throughout the year. This is strongly recommended because the monitoring of bacterial contamination based on testing for faecal coliforms in bivalves or water samples is neither sufficient nor effective.

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