A Preliminary Study of Chloramphenicol Residues in Frozen Raw Shrimps from Processing Plants in Sarawak

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Abstract: A total of 221 (200-500 g/sample) of frozen raw shrimps (*Penaeus monodon* and *Penaeus vanammei*) samples from Sarawak's' processing plants were obtained from January to September 2007 for determination of chloramphenicol residues using Liquid Chromatography Tandem Mass Spectrometry (LC-MS/MS). Out of 221 frozen shrimp samples examined, residues of chloramphenicol was detected in 183 samples (82.8%) ranging from 0.001-6.283 ng/ml. A total of 36 (16.3%) positive samples recorded level above the safety limit or MRPL (0.3 ng/ml) as proposed by the European Union for safe consumption. This study demonstrates that although banned, chloramphenicol might still being used in shrimp farms in Sarawak.

Keywords: chloramphenicol-residues, LC-MS/MS, shrimps, processing plants

Abstrak: Sejumlah 221 sampel (200-500 g/sampel) udang sejuk beku (*Penaeus monodon* dan *Penaeus vanammei*) dari kilang pemprosesan udang di sekitar Sarawak telah diperolehi daripada Januari hingga September 2007 untuk menentukan residu chloramphenicol di dalamnya dengan menggunakan Liquid Chromatography Tandem Mass Spectrometry (LC-MS/MS). Daripada sejumlag semua sampel 221 sampel udang yang telah dianalisis, residu kloramfenikol ditemui dalam 183 sampel (82.8%) dari julat 0.001-6.283 ng/ml. Residue kloramfenikol dalam 36 sampel (16.3%) sampel melebih Minimum Required Performance Limit (MRPL) 0.3 ng/ml, piawaian keselamatan yang telah ditentukan oleh Kesatuan Eropah (EU). Kajian ini menunjukkan bahawa kloramfenikol mungkin masih digunakan dalam industri ternakan udang di Sarawak.

Introduction

Antibiotics are widely used in veterinary practices either as therapeutic (treatment of disease) or subtherapeutic (prevention of disease or improved production) (Anon., 2006) purposes. In addition, they are also widely used as growth promoter in animals (Vaseeharan *et al.*, 2005). The antibiotics are either directly injected into the animals or used as additives in their feeds. Despite all the benefits, problems may occur in the form of residues present in the meat or milk of the animals subjected to such treatment (Vinas *et al.*, 2005). Ingestion of food contaminated with antibiotic residues could pose a health risk to the public. Thus, screening and trace-level quantitation of antibiotics in food products are essential.

One example of widely used antibiotic is chloramphenicol; a broad-spectrum antibiotic used to treat food-producing animals since 1950's (Lucia and Fernando, 2006). Its use however has been associated with toxic effects in human (Goodman *et al.*, 1985) and therefore was banned in 1994 by the European Community (Scortichini, 2005). The use of chloramphenicol in food producing animals, particularly in aquaculture, is also prohibited in the United States. (Anon., 1997). However, this drug is still being used in most of the Asian countries, which are also known to be the greatest producers of seafood (Anon., 2006). In Malaysia, chloramphenicol is prohibited under the Malaysian Food Act (1983) and Food Regulation (1985). However, since it is relatively cheap and readily available in the market, the tendency for chloramphenicol to be misused is rather high (Vinas *et al.*, 2006).

The European Union (EU) has set a Minimum Required Performance Limit (MRPL) of chloramphenicol at 0.3 ng/ml (European Commission, 2003). Seafood exports particularly shrimps have been continuously rejected by importing countries because of the significant amount of chloramphenicol residues. In 2004, two containers of shipment of *Penaeus monodon* from Malaysia were rejected by the

importing countries due to the detection of antibiotic residues in them (Shrimp News International, 2004). This could lead to significant economic consequences for the seafood industry.

Residue of chloramphenicol has been detected in fresh and frozen shrimp from Asia (Storey *et al.*, 2003). The use of liquid chromatography tandem mass spectrometry (LC-MS/MS) was proven as the most selective and sensitive method for chloramphenicol determination in seafood and meat matrices (Storey *et al.*, 2003). Other methods include liquid chromatography-mass spectrometry (Niessen, 1998; Ramos *et al.*, 2003), gas chromatography (Shen and Jiang, 2005), ion trap mass spectrometers (Turnipseed *et al.*, 2003) and atmospheric pressure photoionization (Takino *et al.*, 2003). In Malaysia, works on the detection of chloramphenicol in seafood using LC-MS/MS have just started. Our first work focused on the detection of chloramphenicol residues in freshly harvested tiger shrimps (*Penaeus monodon*) collected from aquaculture farms in Sarawak (Lim and Yong, 2007). The results obtained demonstrated that chloramphenicol residues were detected in 13% of the samples tested. This suggests that chloramphenical could be moderately used in Sarawak's shrimp farms.

Given that there is still limited information regarding the misuse of chloramphenical in our shrimp culture practices, this study continues to determine the presence of chloramphenicol in fresh shrimp, and this time from processing plants in Sarawak. Since the source of shrimps in these plants is mainly from cultured shrimp from the surrounding areas, the results obtained could confirm the earlier observations which suggest that chloramphenicol is still being used in Sarawak's shrimp farms.

Materials and methods

Shrimp samples

A total of 221 samples of frozen raw shrimps (*P. monodon* and *P. vannamei*) (200-500 g/samples, comprised of ~ 20 tails of ± 20 g per shrimp) from different private processing plants around Sarawak were obtained from January to September 2007. No samples were examined in the month of July. A total of 131 samples were obtained from Plant A, 42 from Plant B, 10 from Plant C, 8 from Plant D, 20 from Plant E, 8 from Plant F and 2 from Plant G. Plan A is a very big processing plant located near to Fisheries Research Institute, Bintawa and is also one of Sarawak's main exporters of tiger shrimps. The daily production of this plant is very high thus more samples were taken. Only 2 samples were collected from Plant G which is very small in size and production and located in a district.

Sample preparation

The heads, shell and body appendages of the shrimp samples were removed. Shrimp flesh/tissue was washed under running water before being homogenized in a blender. The samples were analyzed in triplicate. Five grams of homogenized samples was placed in a 50 ml centrifuge tube. The coded mix matrix samples (MMS) of 0, 0.1, 0.2, 0.5, 1.0 and 2.0 ng/ml (spiked samples) were prepared and added with 0, 20, 40, 100, 200 and 400 µl of 25 ng/ml standard chloramphenicol solution. The blanks, MMS and unknown samples were fortified with 50 µl of 50 µg/l chloramphenicol-D_5 (ISTD). The blank and MMS samples were from blank tested tiger shrimp samples. After 20 min equilibration at room temperature, the samples were added with 10 ml of water, vortexed for 1 min and centrifuged for 15 min at 2700 g. Three milliliters of the supernatant was transferred into the Extrelut® NT3 column with no conditioning. The mixture was then eluted with 15 ml of dichloromethane (Merck, Darmstadt, Germany). The solution was evaporated until dry and 0.5 ml of water was added to reconstitute it, followed by 2 ml of toluene (Merck, Darmstadt, Germany). The solution was mixed and centrifuged again. Three hundred microlitres of the aqueous phase (lower phase) of the samples and the MMS were transferred into LC-MS/MS vials. The mixed matrix recovery standard (MMRS) samples of spiked level of 0.2 ng/ml and 0.5 ng/ml (fortified earlier with 50 µl of 50 µg/l ISTD) were prepared by transferring the exact amount of sample extract and standard chloramphenicol solution of 11 µl and 28 µl with 239 µl and 222 µl of water added respectively. The overview of the procedure is illustrated in Fig. 1.

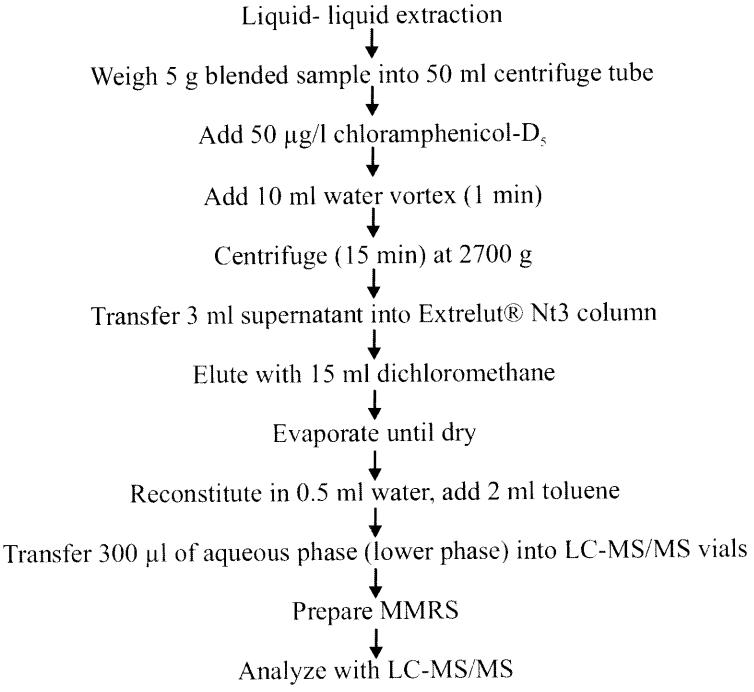

Figure 1: Flow chart of the general overview of chloramphenicol extraction and detection

Reagents

LC column (Hypersil Gold, Thermo, USA) consisted of Inertsil ODS-3 (2.1 mmx50 mmx3 µm) with guard column (2.1 x 10 mm) of the same material and liquid-liquid phase extraction with Extrelut® NT3 (Merck, Darmstadt, Germany) material were used. Acetonitrile, methanol and water were all HPLC grade solvents (Merck, Darmstadt, Germany). Chloramphenicol was supplied by Cambridge Isotope Lab Inc. (Andover, USA). Chloramphenicol-D_5 was used as internal standard (ISTD). The working solution of ISTD was prepared by diluting chloramphenicol-D_5 to 0.1 ng/ml in Methanol-Water (1:1) (Merck, Darmstadt, Germany).

Electron Spray Ionization-Double Mass Spectrometry (ESI-MS-MS)

A quantum triple stage quadruple (TSQ) instrument, together with Surveyor LC quaternary pump and autosampler (Thermo Finnigan, San Jose, USA), were used to detect chloramphenicol in the tissue of tiger shrimps. The instrument was optimized for chloramphenicol using automated optimization procedure in syringe infusion mode as described by the manufacturer. The Multiple Reaction Monitoring (MRM) transitions of particular compounds were monitored. LC-MS/MS separation was done on Inertsil ODS-3, 2.1 x 50 mm, 3 µm column in acetonitrile:water (20:80) at flow rate of 200 µL/min in negative ionization mode. A selected reaction monitoring (SRM) procedure was applied and m/z 321>152 (quantifier), 321>194, 321>257 (qualifiers), 326>157 (IS) transitions were monitored with a total run time of 8 min. Ion ratio of the above masses was monitored throughout the analysis.

Results

In this study, an optimization procedure were performed using syringe infusion of chloramphenicol similar to the operation of the LC-MS/MS described by Bogusz *et al.* (2004) and Vinas *et al.* (2006). The full scan spectrum of chloramphenicol and the product scan of the ion *m/z* 321 were detected and monitored. The MS response to chloramphenicol was found to have a limited linear range. To quantitate levels at around 0.1 ng/ml, a linear standard curve from 0 to 1 ppb was used. This curve gave correlation coefficients (R) of

>0.9939. The retention time at 3.75 minutes was quite stable. A standard curve was plotted for every analysis. Fig. 2 shows an example of a MS response to chloramphenicol standard (a linear curve with equation y=0.421033x + 0.0636195 and R^2 = 0.9939) done in the month of February 2007. R^2 is the correlation factor between the ratio of the area and the concentration of the chloramphenicol standard. The standard curve was determined at every month of analysis and the R^2 values obtained is always near 1.000. This indicates that the LC-MS/MS used in this study was stable and giving an accurate results.

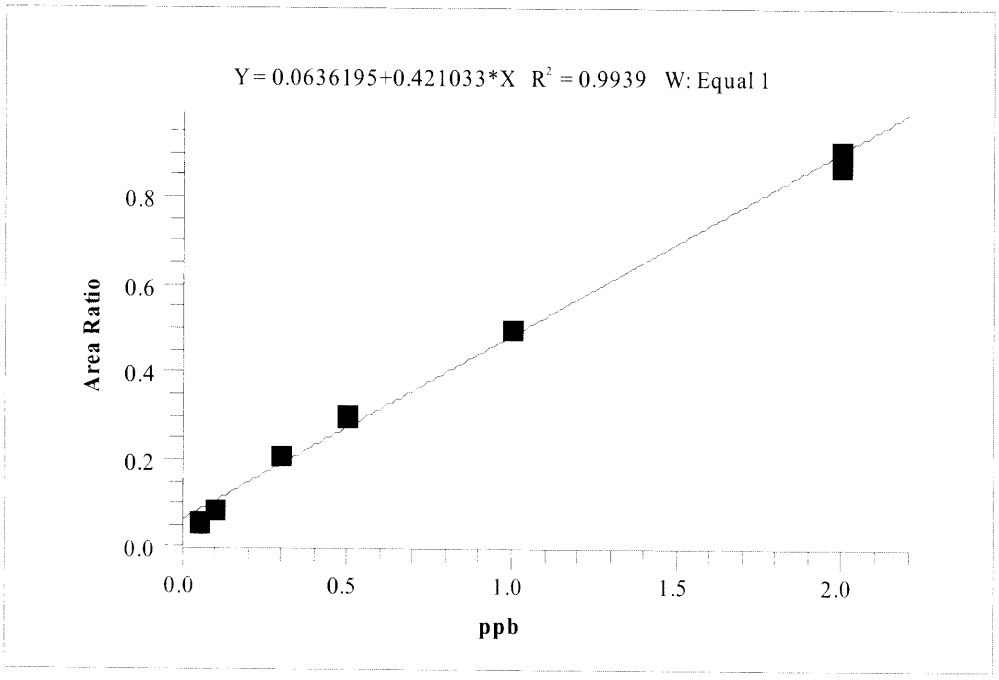

Figure 2: An example of MS response to chloramphenicol standard having linear curve with y = 0.421033x + 0.0636195 and R^2 = 0.9939 done in February 2007

Out of 221 frozen shrimp samples examined, residues of chloramphenicol were detected in 183 samples (82.8%) ranging from 0.001-6.283 ng/ml. A total of 36 (16.3%) positive samples recorded level above the safety limit or MRPL of 0.3 ng/ml. The detailed results of chloramphenicol residues in frozen shrimp samples from selected plants in Sarawak during the survey period are illustrated in Fig. 3 and explained in the following paragraphs.

A total of 131 frozen shrimp samples were tested from Plant A in February (33), March (28), May (16), June (18), August (20) and September (16). Residues of chloramphenicol were detected in 118 shrimp samples with concentrations ranging from 0.008-6.283 ng/ml. In February, all shrimp samples recorded concentrations below the MRPL (0.008-0.252 ng/ml). In contrast, 7 out of 28 (25%) shrimp samples collected in March recorded chloramphenicol concentrations above the MRPL (0.530-6.383 ng/ml). This constitutes about 1.8-21.3 fold higher than the acceptable limit (0.3 ng/ml). Only 1 sample recorded chloramphenicol concentration above MRPL in May (0.445 ng/ml) and June (0.484 ng/ml). More shrimp samples with high concentrations of chloramphenicol were detected in Aug (20%) and Sep (12.5%) ranging from 0.303-0.570 ng/ml and 0.536-0.748 ng/ml respectively. Chloramphenicol was not detected in 13 shrimp samples (7 from February 2007, 4 from March 2007 and 2 from June 2007).

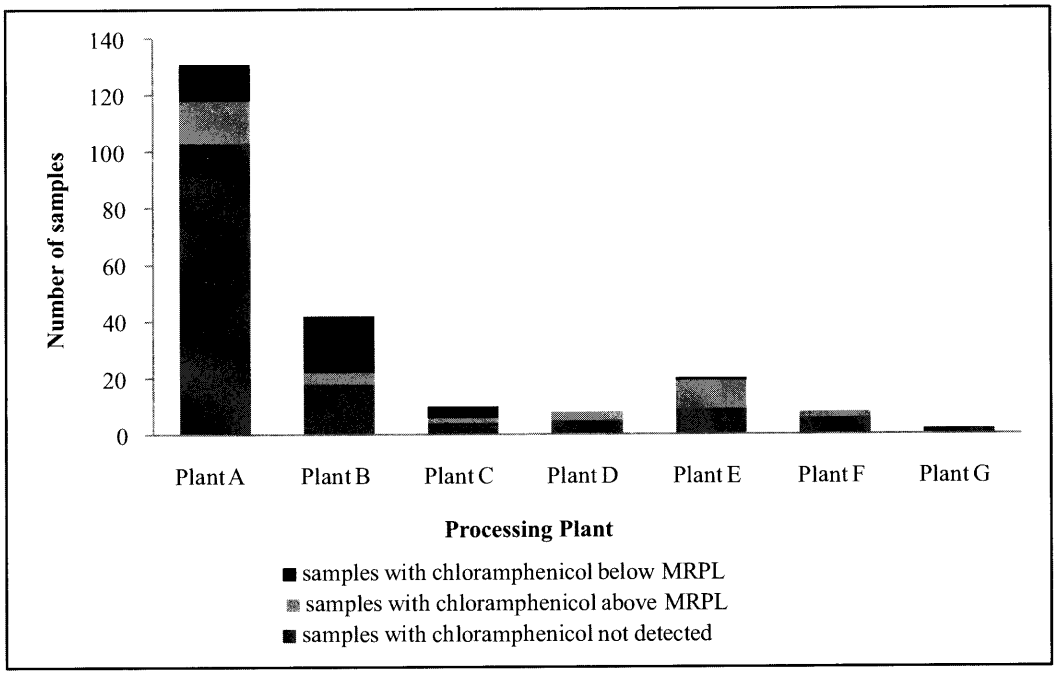

Figure 3: The results of chloramphenicol residues examination in shrimp samples from seven plants from January to September 2007

From plant B, a number of 42 shrimp samples were analysed in January (16), Feb (14), March (10) April (2) and May (2). Chloramphenicol residues were detected in 22 (52.4%) shrimp samples with concentrations ranging from 0.015 1.427 ng/ml. Chloramphenicol was not detected in majority of the samples collected in Jan 2007 (13 out of 16). Only 3 shrimp samples were found to be contaminated with chloramphenicol, however at a very low concentration (0.049-0.059 ng/ml). Similarly in February, 6 out of 12 samples were free from chloramphenicol while others recorded low level of residues (0.015-0.256 ng/ml). In March however, 2 out of 10 (20%) samples contained chloramphenicol concentrations above the MRPL (0.311-1.052 ng/ml) which is about 1.0-3.5 fold higher than the acceptable limit of 0.3 ng/ml. Low level (0.109 ng/ml) of chloramphenicol was detected in 1 out of 2 samples collected in April while both samples examined in May recorded chloramphenicol above the MRPL (1.230-1.427 ng/ml).

Only 10 shrimp samples were analyzed from Plant C (6 and 4 in February and April respectively). In February, only 2 (33.3%) shrimp samples were positive with chloramphenicol at concentration below the MRPL (0.001-0.003 ng/ml) while the rest of the samples were free from it. In April, all shrimp samples were positive with chloramphenicol with 2 out of 4 (50%) of the samples recorded levels above the MRPL (0.367-0.405 ng/ml). This constitutes about 1.20-1.35 times higher than acceptable limit of 0.3 ng/ml.

Compared to other plants, samples from Plant D (n= 8) were only carried out in March 2007. Chloramphenicol was discovered in all shrimp samples with concentrations ranging from 0.019-4.288 ng/ml. A number of 3 samples from this plant (37.5%) recorded chloramphenicol concentrations above the MRPL (0.471-4.288 ng/ml). This represents about 1.5-14.2 fold higher than the acceptable level.

For Plant E, a total of 20 frozen shrimp samples were analyzed in January and March. In January, 4 out of 8 (50%) of shrimp samples recorded chloramphenicol levels above the MRPL (0.302-0.650 ng/ml) which is about 1.0-2.1 times higher than acceptable limit of 0.3 ng/ml. In March, high concentrations of chloramphenicol (in 50% of the samples) ranging from 0.336-1.728 ng/ml were also noted from shrimps obtained from this plant.

A total of 8 frozen shrimp samples were tested from Plant F in March and August. Residues of chloramphenicol were detected in all shrimp samples from this plant ranging from 0.056-1.235 ng/ml. In March, 2 out of 5 (40%) of shrimp samples collected recorded chloramphenicol concentrations above the MRPL (1.107-1.235 ng/ml). This is about 3.69-4.1 fold more than the acceptable limit. Chloramphenicol was still detected in August however at low concentration, below the MRPL (0.093-0.165 ng/ml).

Finally only 2 shrimp samples were analyzed from Plant G in August. Both samples recorded chloramphenicol level below the MRPL (0.084-0.105 ng/ml).

Fig. 4 illustrates the prevalence of chloramphenicol residue during the study period (January until September 2007). Generally chloramphenicol was detected in shrimp samples throughout the sampling period. The highest prevalence of chloramphenicol residue in shrimp samples was noted in March followed by February, August and January. The prevalence of chloramphenicol in shrimp samples was lowest in April.

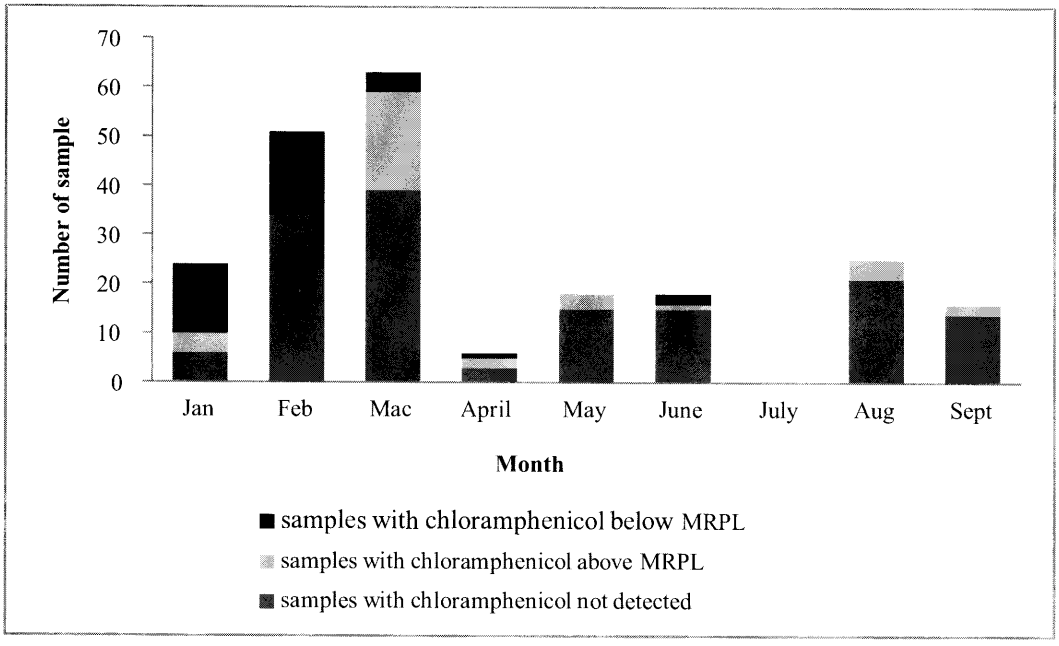

Figure 4: Prevalence of chloramphenicol residue in the shrimp samples during the study period

Discussion

At present, there is very limited data on the detection of chloramphenicol residues in food products including shrimp especially in Malaysia. This study is the first to report on the prevalence of chloramphenicol residues in frozen fresh shrimps from selected processing plants in Sarawak.

Residues of chloramphenicol were detected in 183 samples (82.8%) ranging from 0.001-6.283 ng/ml. From this, 36 (16.3%) shrimp samples also recorded level above the safety limit or MRPL of 0.3 ng/ml. Lu *et al.*, (2009) who used the same method and LC-MS/MS also detected almost similar ranges of chloramphenicol residues (0.301-1.50 ng/ml). in tissues of grass carp, carp and chub. In addition, Tittlemier *et al.*, (2006) who analysed thirty samples of shrimp, marine fish, freshwater fish and canned fish in Canada detected similar levels of about 0.40 ng/ml of chloramphenicol residue in one of the samples examined. The source of chloramphenicol in shrimp culture practices could be from direct injection or mixed with feed.

Flaherty *et al.* (2000) reported that most commercial shrimp feeds are enriched with antibiotics. However there has not been any study on the presence of chloramphenicol in commercial or manually prepared feeds in in Malaysia or Sarawak specifically.

The data obtained in this study reveals a very high prevalence of chloramphenicol residues in frozen shrimp samples from processing plants around Sarawak. The processing plants involved might have used locally cultured shrimps as their raw materials. This indicates that local shrimp farmers are still using chloramphenicol in their shrimp culture although it is banned. This statement is strengthened by our earlier findings which demonstrated that 13.0% of the fresh shrimp sampled from shrimp farms around Sarawak farms are contaminated with the same antibiotic (Lim and Yong, 2007). Our previous findings also noted most of the positive samples originated from large and intensively operated farms. On the other hand, the processing plants could have also used imported contaminated shrimps as their raw materials.

There was no distinguished pattern or trend in the prevalence of chloramphenicol residues in shrimp samples observed in this study. Chloramphenicol was detected throughout the sampling period. The prevalence and the number of samples exceeding the safety limit were highest in the month of March followed by February. The prevalence dwindled in other months. One possibility could be that the farmers were using the chloramphenicol without proper guidance and knowledge. They could have given the antibiotics to shrimps without proper schedule, thus affecting the withdrawal times of the antibiotic. The amount of antibiotic given could also vary. This is why in some months the shrimps contained more chloramphenicol residues than the other months. The procedures of sampling and handling could also affect the results if the shrimps were handled using bare hands by workers using chloramphenicol themselves for treatment of eye, ear or skin infection (Saw, 2007). This might yield to low level detection of chloramphenicol in shrimps as observed in many samples tested in this study.

The results obtained in this survey confirmed that chloramphenicol is being widely used in most of the shrimp farms in Sarawak despite the claim by most of the farmers that they did not used any antibiotics in their practices. Similarly, Lu *et al.* (2009) also insinuated that although chloramphenicol was banned in China, the amount of chloramphenicol observed in the fish samples indicated that the misuse of chloramphenicol in the aquaculture practices in Guangzhou, China was serious. Moreover 74% of the farmers interviewed in Thailand admitted to using antibiotics in their shrimp pond management (Holmstrom *et al.*, 2003).

This study also demonstrates the usefulness of LC-MS/MS in detecting the low level of chloramphenicol residues in shrimp tissues. This method could be use as effective and sensitive tools to monitor and detect the illicit use of chloramphenicol in shrimp production. In the future, more samples should be examined including feeds and pond water. The details of the processed shrimps such as their weigh, their origin, their age, the feed used etc should be taken into consideration when collecting samples. This will give clearer picture of the problem.

Conclusion

This is considered the baseline study conducted in the processing plants in Sarawak. Although no comparative analysis could be derived because of inconsistency in the number of samples, this study suggest that chloramphenicol might still being used in shrimp farms in Sarawak. The imported shrimp with the purpose of re-exporting could also be contaminated with chloramphenicol.

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References

Anon., 1996. The Merck Index, 12th Edition, 1996.

Anon., 1997. GESAMP Reports and Studies No.65, Rome 1997.

Anon., 2006. Antibiotic in animal feeds. Eastshire communications Newsletter 23 Jan.2006

Anon., 2006. The rise and fall of chloramphenicol Shrimp News International (August 2001 through May 2006). p 44.

Commission Decision, 2003. 13 March 2003. Amending Decision 2002/657/EC as regards the setting of minimum required performance limits (MRPLs) for certain residues in food of animal origin (2003/181/EC), *Official Journal of European Community*, **L71**: 17-18.

H., Al-Enazi, E., Ibrahim, Z. and Al-Tufail, M. 2004. Rapid determination of chloramphenicol and its glucuronide in food products by liquid chromatography-electrospray negative ionization tandem mass spectrometry. *Journal of Chromatography B*, **807**: 343-356.

European Commission 2003. Official Journal of the European Union L 71/17, 15 March 2003.

Flaherty, M., Szuster, B. and Miller, P. 2000. Low salinity inland shrimp farming in Thailand. *Ambio*, **29**, 174-179.

Akta Makanan (1983) dan Peraturan-Peraturan Makanan 1985. MDC Penerbit Pencetak Sdn. Bhd.

Goodman, G.A., Goodman, L.S., Rall, T.W. and Murad, F. 1985. The Pharmacological Basis of Therapeutics. 7thSeventh Ed., Mac-Millan, New York.

Holmstrom, K., Graslund, S. Wahlstrom, A., Poungshompoo, S. Bengtsson, B.E. and Kautsky, N. 2003. Antibiotic use in shrimp farming and implications for environmental impacts and human health. *International Journal of Food Science and Technology*, **38**: 255-266.

Lim, M.H. and Yong, A.H. 2007. Determination of chloramphenicol residues in tiger shrimps from aquaculture farms in Sarawak by electrospray triple quadrupole liquid chromatography tandem mass spectrometry (LC-MS/MS). 423-433 pp. *In* Chee, P.E. *et al.*, (Eds). *Proceedings of the 4th National Fisheries Symposium 2006*. Advancing R&D towards fisheries business opportunity, Kuching. 488 pp.

Lu, X.W., Dang, Z. and Yang, C. 2009. Preliminary investigation of chloramphenicol in fish, water and sediment from freshwater aquaculture pond. *International Journal of Environmental Science and Technology*, **6 (4)**, 597-604.

Lucia, S. and Fernando, R. 2006. Analytical methodologies for chloramphenicol residues determination in food matrixes: A Brief Review. Current Pharmaceutical analysis. Bentham Science Publishers. **2(1)**, February 2006, 53-57(5) pp.

Niessen, W.M.A. 1998. Analysis of antibiotics by liquid chromatography-mass spectrometry. *Journal of Chromatography A*, **812**: 53-75

Ramos, M., Munoz, P., Aranda, A., Rodriguez, I., Diaz, R. and Blanca, J. 2003. Determination of chloramphenicol residues in shrimps by liquid chromatography-mass spectrometry. *Journal of Chromatography B*, **791**:31-38.

Saw H.Y. 2007. Contamination problems in Aquaculture Chemotherapeutic drugs. JTF II Regional workshop in seafood safety testing for Laboratory Personnel, 2-5 October 2007, Singapore. p.2.

Scortichini, G., Annunziata, L., Haouet, M.N., Benedetti, F., Krusteva, I. and Galarini, R. 2005. ELISA qualitative screening of chloramphenicol in muscle, eggs, honey and milk: method validation according to the Commission Decision 2002/657/EC/Criteria. *Analytica Chimica Acta*, **535**: 43-48.

Shen, H.-Y. and Jiang, H.-L. 2005. Screening, determination and confirmation of chloramphenicol in seafood, meat and honey using ELISA, HPLC-UVD, GC-ECD, GC-MS-EI-SIM and GCMS-NCI-SIM methods. *Analytica Chimica Acta*, **535**:33-41.

Storey, J., Pfenning, A., Turnipseed, S., Nandrea, G., Lee, R., Burns, C. and Madson, M. 2003. Determination of chloramphenicol residues in shrimp and crab tissues by electrospray triple quadrupole LC/MS/MS. *Laboratory Information Bulletin,* 4306, 14 p.

Takino, M., Daishima, S. and Nakahara, T. 2003. Determination of chloramphenicol residues in fish meats by liquid chromatography-atmospheric pressure photoionization mass spectrometry. *Journal of Chromatography A,* **1011**:67-75.

Tittlemier, S.A., Van De Riet, J., Burns, G., Potter, R., Murphy, C., Rourke, W., Pearce, H. and Dufresne, G. 2007. Analysis of veterinary drug residues in fish and shrimp composites collected during the Canadian total diet study, 1993-2004. *Food Additives and Contaminants,* **24(1):**14-20.

Turnipseed, S.B., Roybal, J.E., Pfenning, A.P. and Kijak, P.J. 2003. Use of ion-trap liquid chromatography mass spectrometry to screen and confirm drug residues in aquacultured products. *Analytical Chimica Acta*, **483**: 373-386.

Vaseeharan, B., Ramasamy, P., Murugan, T. and Chen, J.C. 2005. *In vitro* susceptibility of antibiotics against *Vibrio* spp. and *Aeromonas* spp. isolated from *Penaeus monodon* hatcheries and ponds. *International Journal of Antimicrobial Agents,* **26**: 285-291.

Vinas, P., Balsalobre, N. and Hernandez-Cordoba, M. 2006. Determination of chloramphenicol residues in animal feeds by liquid chromatography with photo-diode array detection. *Analytica Chimica Acta,* **558**: 11-15.