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Determination of Chloramphenicol (CAP) Residues by LC-MS/MS in Shrimp Available in Commercial Markets of Lahore

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Abstract

Eighty seven samples of shrimps (*Penaeus indicus*) were randomly collected from four major markets of Lahore. The samples were transported to Fish Quality Control Labs Lahore. The shrimps were tested for the presence of chloramphenicol (CAP) on LC-MS/MS using negative ion mode and electron spray source. The CAP was not detected in any of the collected samples. It is concluded that shrimps sold in different markets of Lahore are safe for human consumption.

Keywords: Chloramphenicol, LC-MS/MS, Lahore, FQCL, Shrimps.

INTRODUCTION

In aquaculture, bacterial diseases are serious problem and represent the most important challenge (Morales, 2004; Holmstrom *et al.*, 2003). Antibiotics are commonly used to control the bacterial diseases during the production cycle, both in the larval and growth phase. The accumulation of antibiotic residues in the edible tissues of shrimp may alter human intestinal flora and cause food poisoning or allergy problems (Ma *et al.*, 2006).

Chloramphenicol (CAP), a broad spectrum antibiotic, is widely used in aquaculture against various pathogens, especially to control those which are resistant to other antibiotics (Wang *et al.*, 2014). CAP has been preferred by shrimp farmers for its benefits of sub therapeutic use since it has a long half-life in solution and its optimal activity is between pH 7.4 to 8.0 (Ashwin *et al.*, 2005).

In view of the possibility of transmitting residues of CAP to human beings through aquaculture products, the use of CAP under any circumstances in aquaculture has been banned in many countries including US, EU, India, Thailand and Singapore (Yogeswari *et al.*, 2005). A daily dose of CAP may lead to the development of bone marrow depression (aplastic anaemia) in human beings (Greenwood, 2000; Maluf, 2002; Young, 2002).

The shrimp consumption has increased in recent years. The change shifted towards better understanding of healthy diet and nutritional importance of seafood consumption. Seafood is high protein source, vitamin D, vitamin B3 and zinc being beneficial for health. Among the fisheries sector shrimps growing is most profitable, economical and has great export value. Shrimps contain high protein content, calories, enzymes, vitamins and minerals (Storey *et al.*, 2012).

Shrimps in Pakistan are also being used in human food therefore it is mandatory to check the quality of shrimps, being an item of import and local culture in Pakistan for the presence of banned CAP and heavy metals. Keeping in view their health hazards to humans the present study has been planned to check the CAP quantitatively in shrimps available/being sold in local/commercial markets of Lahore.

MATERIALS AND METHODS

Shrimp (*Penaeus indicus*) samples were collected from Hyper star (Fortress stadium), Metro (Thokar Niaz Baig), Al-Fatah super store (Gulberg) and Main fish market of Lahore. Sampling was performed monthly from November 2017 to June 2018 in triplicate. Each sample was placed separately in plastic bag (stored in ice), identified and delivered to the laboratory for testing.

Methanol, Hexane, 1-Propanol, Formic acid, Acetonitrile and Anhydrous Sodium Sulphate. Mobile Phase (Acetonitrile: 0.1% formic acid, 2:8). Chloramphenicol stock solution was prepared at concentration 1 mg/ml in mobile phase. The working standard was prepared as per requirement from the stock solution. 10 g of sodium sulphate and 25 ml of acetonitrile were added in the 5g of sample, homogenized and centrifuged for 5 minutes. The upper clear layer of acetonitrile was separated from the residue. 25 ml of acetonitrile was added to the residue, sonicated for 30 sec and then centrifuged for 5 minutes. The upper clear layer was removed and added to the first acetonitrile layer. 10 ml of 1-Propanol added to the acetonitrile layer, evaporated and residue collected with 1 ml of mobile phase into the sample vial (Nair *et al.*, 2008). Instrumentation parameters details are mentioned in table 1.

Table 1. Details of Instrument Parameters

LC System	Thermo Fisher Surveyor LC Pump plus
Column:	Hypersil Gold C ₁₈ , Dia 250x4.6 (mm)
Mobile Phase	Acetonitrile : 0.1 % formic acid (2:8) (Isocratic)
Flow Rate:	0.5 ml/min
Injection Vol	75 µL
MS System:	LCQ Fleet mass spectrometer system
Run time:	15 min
Retention time	10 min
Parent ions:	m/z 321
Ion Source	ESI
Polarity	-ve

QuanBrowser of Xcalibur was used for quantification of Chloramphenicol in standards and samples.

RESULTS AND DISCUSSION

In the present study shrimp species (*Penaeus indicus*) having average weight range 17.2 gm to 35 gm and average length from 9.8 cm to 18 cm were collected from four major commercial markets of Lahore in triplicate for the determination of chloramphenicol. The chloramphenicol analysis was performed by LC-MS/MS following the procedures used by (Nair *et al.*, 2008). The table 2 shows the results of chloramphenicol. Among all eighty seven (87) collected samples no chloramphenicol detected.

Table 2. Concentration of Chloramphenicol in Shrimp

Sampling Month	Sampling Sites	Shrimps Species	No. of Samples	Average Weight (gm)	Average Length (cm)	Conc. of Chloramphenicol
November, 2017	Hyper star (Fortress stadium)	<i>Penaeus indicus</i>	3	17.6	11.2	ND
	Metro (Thokar Niaz Baig)	<i>Penaeus indicus</i>	3	29.2	9.8	ND
	Main fish market of Lahore	<i>Penaeus indicus</i>	3	25.0	12.1	ND
December, 2017	Hyper star (Fortress stadium)	<i>Penaeus indicus</i>	3	20.5	11.2	ND
	Metro (Thokar Niaz Baig)	<i>Penaeus indicus</i>	3	17.2	9.8	ND
	Main fish market of Lahore	<i>Penaeus indicus</i>	3	21.0	12.1	ND
January, 2018	Hyper star (Fortress stadium)	<i>Penaeus indicus</i>	3	21.0	12.4	ND
	Metro (Thokar Niaz Baig)	<i>Penaeus indicus</i>	3	23.5	11.7	ND
	Main fish market of Lahore	<i>Penaeus indicus</i>	3	25.0	13.0	ND
February, 2018	Hyper star (Fortress stadium)	<i>Penaeus indicus</i>	3	21.0	11.8	ND
	Metro (Thokar Niaz Baig)	<i>Penaeus indicus</i>	3	19.8	12.5	ND
	Al-Fatah super store (Gulberg)	<i>Penaeus indicus</i>	3	20.7	10.8	ND
	Main fish market of Lahore	<i>Penaeus indicus</i>	3	21.3	12.2	ND
March, 2018	Hyper star (Fortress stadium)	<i>Penaeus indicus</i>	3	21.7	14.0	ND
	Metro (Thokar Niaz Baig)	<i>Penaeus indicus</i>	3	35.0	18.0	ND
	Al-Fatah super store (Gulberg)	<i>Penaeus indicus</i>	3	21.5	12.0	ND
	Main fish market of Lahore	<i>Penaeus indicus</i>	3	23.8	14.2	ND
April, 2018	Hyper star (Fortress stadium)	<i>Penaeus indicus</i>	3	21.5	13.0	ND
	Metro (Thokar Niaz Baig)	<i>Penaeus indicus</i>	3	22.1	14.0	ND
	Al-Fatah super store (Gulberg)	<i>Penaeus indicus</i>	3	23.0	14.2	ND
	Main fish market of Lahore	<i>Penaeus indicus</i>	3	35.0	18.0	ND
May, 2018	Hyper star (Fortress stadium)	<i>Penaeus indicus</i>	3	23.1	14.0	ND
	Metro (Thokar Niaz Baig)	<i>Penaeus indicus</i>	3	24.2	15.0	ND
	Al-Fatah super store (Gulberg)	<i>Penaeus indicus</i>	3	35.0	18.0	ND
	Main fish market of Lahore	<i>Penaeus indicus</i>	3	24.0	15.0	ND
June, 2018	Hyper star (Fortress stadium)	<i>Penaeus indicus</i>	3	21.0	12.0	ND
	Metro (Thokar Niaz Baig)	<i>Penaeus indicus</i>	3	21.5	11.4	ND
	Al-Fatah super store (Gulberg)	<i>Penaeus indicus</i>	3	25.0	13.0	ND
	Main fish market of Lahore	<i>Penaeus indicus</i>	3	21.0	12.0	ND

ND: None detected

The mass spectrometer was operated in the negative ion mode using selected reaction monitoring and the precursor ion at $m/z = 321$ yielded four main product ions of $m/z = 257, 194, 176$ and 152 . The peak area of the $m/z = 152$ peak was used for the quantification linear plots obtained between 0.50 and 10.0 ng/ml CAP.

Ma et al. (2006) studied the effect of Chloramphenicol on microorganism in aquaculture the results showed that high concentration of Chloramphenicol could inhibit the activities of microorganisms in sediment. Many shrimp culture formers are not well informed about the efficient and safe application practices of chloramphenicol and other antibiotics. A more restrictive use of antibiotic could have positive effect for the individual farmers and

simultaneously, decrease impacts on regional human medicine and adjacent coastal ecosystem (Holmstrom *et al.*, 2003).

In the present study the chloramphenicol was not detected from any samples among all the samples collected from the major markets of Lahore. Our current studies agrees with the results of Neuhaus *et al.* (2009) who found that all the samples collected from wild and aquaculture farms in three states of India (Andhra Pradesh, Tamil Nadu and Kerala) were free from CAP and safe for human consumption.

CONCLUSION

It is concluded that the shrimp being sold at different major commercial markets of Lahore are safe for use as chloramphenicol is absent and heavy metals are within safe limits.

CONFLICT OF INTEREST

The authors declare that no competing interests exist.

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