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Sources and fate of antimicrobials in integrated fish-pig and non-integrated tilapia farms

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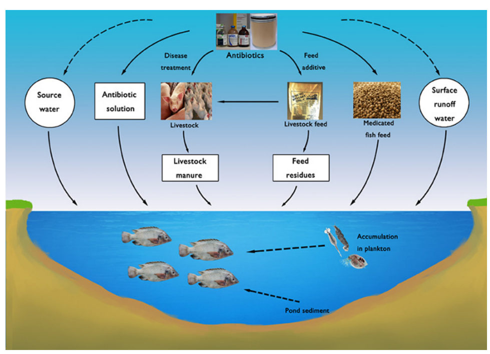
HIGHLIGHTS

- Residues of 11 antimicrobials were investigated from tilapia-pig integrated and non-integrated farms.
- Enrofloxacin and sulfadiazine were the most commonly found antimicrobials in integrated farms whereas sulfadiazine was commonly found from non-integrated farms.
- Concentration of antimicrobial residues differed in tilapia fish skin and muscle.
- Antimicrobial residues in pig manure are not associated with residues found in tilapia.
- Both fish feed and pig feed are not the source of antimicrobial contamination in fish pond.

GRAPHICAL ABSTRACT



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ABSTRACT

Antimicrobial contamination in aquaculture products constitutes a food safety hazard, but little is known about the introduction and accumulation of antimicrobials in integrated fish-pig aquaculture. This study, conducted in 2013, aimed to determine the residues of 11 types of antimicrobials by UPLC-MS/MS analysis in fish feed ($n = 37$), pig feed ($n = 9$), pig manure ($n = 9$), pond sediment ($n = 20$), fish skin ($n = 20$) and muscle tissue ($n = 20$) sampled from integrated tilapia-pig farms, non-integrated tilapia farms and fish feed supply shops. There was a higher occurrence of antimicrobial residues in fish skin from both integrated and non-integrated farms, and in pig manure. Enrofloxacin (3.9–129.3 μg/kg) and sulfadiazine (0.7–7.8 μg/kg) were commonly detected in fish skin and muscle, pig manure and pond sediment from integrated farms, with different types of antimicrobials found in pig manure and tilapia samples. In non-integrated farms, sulfadiazine (2.5–89.9 μg/kg) was the predominant antimicrobial detected in fish skin and muscle, fish feed and pond sediment. In general, antimicrobials seemed not to be commonly transmitted from pig to fish in tilapia-pig integrated farms, and fish feed, pig feed and pond sediment did not seem as important sources of the antimicrobials found in fish from both systems. The frequent findings of antimicrobial residues in fish skin compared with fish muscle was probably due to different pharmacokinetics in different tissue types, which have practical food safety implications since antimicrobial residues monitoring is usually performed analyzing mixed skin and fish muscle samples.

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1. Introduction

Integrated aquaculture is an ancient aquaculture practice in China and has been introduced into other South and South-east Asian countries like Bangladesh, India, Indonesia and Vietnam. These culture systems are mainly practiced by small-scale, often family owned, farms that keep livestock such as pigs or poultry in units located either above the pond or on the pond dyke, allowing drainage of manure, urine and excess feed into the pond, where the nutrients act as fertilizer and facilitate the growth of various kinds of algae and other organisms serving as fish feed. Integrated aquaculture systems are seen as resource efficient and sustainable food production systems providing income for many farmers (Bostock et al., 2010; Little and Edwards, 1999). However, the excessive feed and in particular faecal wastes from the livestock contain a number of substances and microorganisms that are potential hazards to food safety and pond ecology. Livestock faecal matter may contain pathogens that can survive and be transmitted to fish, e.g. zoonotic viruses like Hepatitis E, bacterial pathogens like *Campylobacter* spp. and *Salmonella* spp. and parasites like fishborne zoonotic parasites (Li et al., 2013; Li et al., 2016; Sapkota et al., 2008; Taylor et al., 2013; Xue et al., 2007). A study in Vietnam reported that fish raised in fish-pig ponds contained 100 times higher concentrations of faecal bacteria (*Escherichia coli*), than fish from ponds with no intentional faecal inputs (Dang and Dalsgaard, 2012). Additionally, antimicrobials are routinely added to pig feed to prevent and control diseases that could enter integrated livestock-fish ponds through feed residues and animal wastes (Zhu et al., 2013). As a result, bacteria in the ponds may develop resistance because of selective pressure from exposure to antimicrobials (Zhu et al., 2013). Moreover, antimicrobial residues in fish pond water and sediment exert such a selective pressure even at concentrations below the bacteria's Minimum Inhibitory Concentrations (MIC) values (Xiong et al., 2015). Thus such low concentrations of antimicrobials are likely to play an important role in the maintenance and spread of resistance genes (Andersson and Hughes, 2014). A study conducted on integrated fish farms suggested that poultry faeces along with antimicrobial residues from livestock may contribute to a resistance gene pool in aquaculture systems (Shah et al., 2012).

The food safety hazard associated with antimicrobial residues in cultured fish and shrimp is well-documented and the presence of such residues are typically associated with preventive and therapeutic antimicrobial use followed by too short withdrawal periods before harvest (Done and Halden, 2015; Heuer et al., 2009). Farmers limited knowledge of prudent antimicrobial use, use of antimicrobial products of poor quality, homemade medicated feed and withdrawal periods established based on non-scientific experimental studies are all factors that contribute to risks for detection of antimicrobial residues in aquatic produce, in particular in less developed countries (Li et al., 2016; Phu et al., 2015c; Phu et al., 2015d). However, little is known to what extent antimicrobial residues accumulate in fish raised in integrated fish-livestock aquaculture systems and whether such residues originate from antimicrobials provided to the livestock through medicated feed, drinking water or through individual treatments. Moreover, the content and fate of antimicrobials and residues in faecal and liquid livestock wastes, and excessive feed when discharged into the pond environment, are unknown.

Tilapia (especially Nile tilapia, *Oreochromis niloticus*) has become one of the most cultured and internationally traded food fish in the world, with China producing about 40% of the global production in 2013 (FAO, 2014; Zhang et al., 2015). Guangdong province is located in southern China and produces nearly half of the country's tilapia production (Anonymous, 2010; Li et al., 2013). Integrated intensive tilapia-pig aquaculture is commonly practiced by small and medium scale farms in the province (Li et al., 2016). Farmers feed pigs and tilapia with commercial pelleted feed and apply antimicrobials for prevention and control of diseases in the pigs and fish (Li et al., 2016). Recently, antimicrobial residues have increasingly been detected in exported tilapia fillets (FDA, 2015).

This study aimed to determine the sources and fate of antimicrobials in integrated tilapia-pig and non-integrated tilapia farms and evaluate associated food safety hazards.

2. Materials and methods

2.1. Farm selection and sampling

A total of 207 integrated and non-integrated Nile tilapia (*O. niloticus*) farms located in Maoming district in Guangdong province, China were previously identified in the EU-funded SEAT project (Sustaining Ethical Aquaculture Trade) (Murray et al., 2013). From these farms, we randomly selected 10 integrated tilapia-pig farms and 10 non-integrated farms culturing tilapia only with no intentional input of pig manure fertilizer. The number of pens and pigs varied significantly between the integrated farms and samples were only collected from the pig pen connected to the sampled fish pond. Each farm was designated an individual identification number.

The field visits and sampling were done between October and November 2013. Five tilapia fish from each pond (287.0 ± 133.9 g average weight) were collected with a cast net by farm workers and each fish was placed in a separate sterile plastic bag. At the integrated farm, five representative pig faecal samples (each with a weight of about 50 g) were collected from different locations in the pig pen and mixed into one composite sample which was stored in a sterile plastic bag. Sediment (2–4 cm top sediment of approximately 50 g) was collected with a customized grab sampler from five representative sites in the pond including locations where animal wastes were discharged from the pig pen, and mixed into one composite sample as well. Commercial pelleted fish feed and pig feed (only from integrated farms) samples (approximately 200 g) were collected from feed storage facilities on the farm and placed in sterile plastic bags. Additionally, with the aim to further investigate antimicrobials in commercial feed products, 18 commonly used brands of tilapia feed (approximately 100 g) were sampled from four local feed supply shops.

All samples were transported by car to the laboratory in an insulated box with cooling elements within 18 h of sampling. Since tilapia may be consumed as a whole fish including the skin (e.g. in fried dishes) or as fillets without skin, skin and muscle samples were analysed separately. The fish were dissected in the laboratory, then 5 g of muscle and 5 g of the skin of each tilapia was sampled, each sample type of all the five tilapia from one pond were mixed into composite samples. All samples including fish feed, pig feed, pig manure, pond sediment, tilapia skin, and muscle were subsequently stored at -20 °C with antimicrobial residue analysis initiated within 12 months.

A questionnaire survey was conducted with farm owners during the field visits to obtain information about other potential sources of antimicrobials than medicated feed and disease treatment events. Such sources included pond water and sediment (e.g. accumulated antimicrobials) as well as how these were discharged and reused.

2.2. Antimicrobial residues determination with UPLC-MS/MS

2.2.1. Chemicals and reagents

A total of 11 antimicrobials were chosen and analysed for based on findings reported in a previous study on chemical usage on tilapia farms in China (Li et al., 2016). The reference standards (purity >98%): amoxicillin (AMX), doxycycline (DOX), tetracycline (TET), oxytetracycline (OTC), florfenicol (FFN), sulfadiazine (SDZ), trimethoprim (TMP), enrofloxacin (ENR), epioxytetracycline (EOTC), nalidixic acid (NAL) and ciprofloxacin (CIP), were purchased from Dr. Ehrenstorfer (Augsburg, Germany). As EOTC is the metabolite of OTC in animals, the concentration of the two compounds was reported combined. The