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Occurrence Rates of Sulfamethoxazole and Erythromycin-Resistant Bacteria and Drug Concentrations in Wastewater of Integrated Aquaculture-Agriculture (VAC) Sites in Northern Vietnam

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Abstract—Drug contamination selects for drug-resistant bacteria in agriculture products, animal and human bodies and the environments. Relationship between occurrence of drug-resistant bacteria and the drug contamination in integrated aquaculture-agriculture system (VAC) in Vietnam has not been widely reported. In this study, sulfonamides and erythromycin concentration in water samples collected from fish ponds of VAC sites in July 2007 were measured. Sulfamethoxazole (SMX) was found in all water samples of VAC farms ranging from 68.20 ng/l to 326.00 ng/l in pig-VAC farms, 6.14 ng/l in duck-VAC farm, 3.22 ng/l in duck+pig-VAC farm. Another sulfadrug, sulfamethazine (SMT) was detected with high concentration in duck+pig-VAC farm (1966 ng/l), followed by pig-VAC farms (6.78–851 ng/l), but not detected in duck-VAC farm. Erythromycin was detected in pig-VAC farms (nd–26.6 ng/l) and in duck+pig-VAC farm (3.7 ng/l). No drug-contamination was found in the control site, where no animal was reared. Number of total cultivable bacteria ranged from 10^3 to 10^5 CFU/ml. Percents of SMX^r bacteria was found to be 16.90–31.44% in pig-VAC farms, and 42.60% in duck+pig-VAC farm. Occurrence rates of SMX^r bacteria in VAC farms (5 sites) ranged from 12.8 to 42.6%, whereas in the control site the value was only 4.4%. Considering all the data together, no significant correlation was observed between SMX concentration and occurrence rate of SMX^r bacteria (r^2 = 0.0158), whereas cross resistance to SMT showed a significant relationship (r^2 = 0.8251). ERY^r bacteria also showed positive correlation (r^2 = 0.5441) with ERY concentration. These findings suggest that, although drug contamination in VAC accelerates drug-resistant bacteria, the one-to-one correlation was not clear. Other selective pressure(s) might play as causative agents for drug resistance.

Keywords: drug, contamination, VAC, Vietnam

Table 1. Characteristics of sampling sites and wastewater samples.

Site ID	Characteristics	Water temperature (°C)	Air temperature (°C)	pH
pig-VAC 1	70 pigs	34	33.6	7.58
pig-VAC 2	50 pigs	34	35	7.81
pig-VAC 3	75 pigs	34	33	7.7
duck-VAC	>100 ducks	35	34	9.10
duck+pig-VAC	Combination of 15–20 pigs and >100 ducks	35	36.2	8.65
Control	Lake without animal farm	34	36.2	7.8

INTRODUCTION

Integrated aquaculture-agriculture (VAC) system has become a common farming system in South-east Asian countries over decade. It is considered as an economical method of farming, as it requires only low input and involves efficient use of resources. This system involves animals, often chicken and pigs in cages located above or adjacent to fish ponds. Animal manure is introduced into the ponds, which then will be directly eaten by the fish or remain in ponds and stimulates the growth of planktonic organisms that are then eaten by the fish (Little and Muir, 1987). Chicken and pigs are normally reared intensively in this system by the use of commercial feed supplemented with antibiotics used for growth promotion and prevention of diseases. The animal manure and spilled feed release residual antibiotics directly to the fish pond. This creates a selective pressure on the aquatic drug-resistant bacteria (Petersen and Dalsgaard, 2003).

Recently, high prevalence of antimicrobial resistant bacteria and the resistance determinants have been detected in fish ponds of integrated aquaculture-agriculture (chicken-fish farms) in Thailand (Petersen and Dalsgaard, 2003). However, VAC in Vietnam is different from that in Thailand and drug usage is suspected to be different. This study was carried out to provide information on the correlation between occurrence rates of drug-resistant bacteria and the drug concentrations in aquatic environment of VAC in Vietnam.

MATERIALS AND METHODS

Study sites and sample collection

Water samples were collected in VAC farms (Table 1). In village A, intensive pig farms are dominant, where 50–100 pigs were reared in one farm. Three pig-VAC farms (pig-VAC 1, 2, and 3) were investigated. In village B, VAC system was a combination of pigs and ducks (duck+pig-VAC) or ducks only (duck-VAC). One control site, where no farm was found, was also sampled.

Drug concentration analysis

Analyses of sulfamethoxazole (SMX), sulfamethazine (SMT) and

Table 2. Drug concentration and the number of drug-resistant bacteria.

Site	Total cultivable bacteria (CFU/ml)	SMX conc. (ng/l)	SMT conc. (ng/l)	SMX^r bacteria (CFU/ml) (%)	ERY conc. (ng/l)	ERY^r bacteria (CFU/ml) (%)
pig-VAC 1	3.5×10^6	326	851	8.3×10^5 (23.8%)	12.8	4.9×10^5 (13.98%)
pig-VAC 2	1.3×10^5	68.2	685	2.2×10^4 (16.9%)	0.59	1.5×10^4 (11.53%)
pig-VAC 3	9.0×10^4	69.9	6.78	2.4×10^4 (31.44%)	n.d.	1.8×10^3 (2.00%)
duck-VAC	1.3×10^5	6.14	n.d.	1.6×10^4 (12.8%)	n.d.	1.3×10^3 (1.00%)
duck+pig-VAC	6.0×10^4	3.22	1966	2.6×10^4 (42.6%)	3.7	5.6×10^3 (9.30%)
Control	1.6×10^5	n.d.	n.d.	7.0×10^3 (4.4%)	n.d.	1.3×10^3 (0.81%)

n.d.: not detected.

erythromycin (ERY) were performed as previously described (Managaki *et al.*, 2007).

Detection and enumeration of sulfamethoxazole, and erythromycin-resistant bacteria

The colony forming units (CFU) were enumerated by the plate spread method (Nonaka *et al.*, 2000, 2007). One ml of water sample was diluted in 9 ml of phosphate buffered saline (PBS) and then serial 10-fold dilutions were prepared. In this study, nutrient broth (Difco, Detroit, MD, USA) plus 1.5% agar (called NA-nutrient agar) was used. NA was supplemented with 60 μg/ml SMT or ERY. The bacteria that can grow on NA with 60 μg/ml of drug were considered as drug-resistant bacteria Colony numbers were counted after 5 days of incubation at 30°C. Duplicate counting was performed.

Data analysis

Linear regression and Student's *t* test were used to elucidate the correlation between drug concentration and occurrence rate of drug-resistant bacteria among 6 sites investigated.

RESULTS AND DISCUSSION

The contamination of SMX in this study was found in all water samples of VAC farms with concentrations of 68.20–326.00 ng/l in pig-VAC farms, 6.14 ng/l in duck-VAC farm, and 3.22 ng/l in duck+pig-VAC farm. SMT was also detected with high concentration in duck+pig-VAC farm (1966 ng/l), followed by pig-VAC farms (6.78–851 ng/l), but not in duck-VAC farm (Table 2). SMX residues were comparable to other studies in Vietnam and other countries, where

Table 3. Correlation between drug concentrations and drug-resistant bacteria (%) in VAC wastewater.

Resistance to	Drug		
	SMX	SMT	ERY
SMX	$r^2 = 0.0158, P > 0.05$	$r^2 = 0.8251, P < 0.05$	$r^2 = 0.0678$
ERY	$r^2 = 0.4528$	$r^2 = 0.4493$	$r^2 = 0.5441, P > 0.05$

maximum concentration of SMX detected in wastewater treatment plant in Switzerland was 1900 ng/l, that in Germany was 9000 ng/l (Göbel *et al.*, 2005) and that in Vietnamese aquaculture was 6.06 mg/l (Le *et al.*, 2005).

SMX-resistant (SMX^r) bacteria were also detected in all water samples of six sites. Total CFU was ranged from 10^3 to 10^6 CFU/ml. SMX^r bacteria in pig-VAC farms showed a range of 16.90–31.44%, whereas duck+pig-VAC farm showed a slightly higher proportion of 42.60%. SMX^r bacteria in control site showed only 4.4%. These proportions (16.9–42.6%) of SMX^r bacteria in this study were higher than these (11.6–21.79%) of SMX^r bacteria in the previous study in aquaculture of Vietnam (Le *et al.*, 2005).

In this study, ERY^r bacteria were found in all water samples of VAC and control sites (Table 2). High percentages of ERY^r bacteria were found in the pig-VAC 1 (13.89%), pig-VAC 2 (11.53%), and duck+pig-VAC (9.30%) sites, whereas ERY concentrations were 12.8, 0.59, and 3.7 ng/l, in the respective sites. In contrast, percentages of ERY^r in duck-VAC farm and control sites, where ERY was not detected, were relatively low (1.0 and 0.81% in duck-VAC and control site, respectively). This finding suggests that high incidence of ERY^r could be related to the presence of ERY in wastewater of VAC ($r^2 = 0.5441$, $p > 0.05$) (Table 3).

The positive correlation between drug concentration and drug resistant bacteria was not always observed. In this study, concentrations of SMX detected from VAC farms were lower than those of SMT, while SMX^r was high in relation to SMT concentration. As a result, no correlation was observed between SMX concentration and occurrence of SMX^r bacteria ($r^2 = 0.0158$, $P > 0.05$), whereas significant correlation between SMT and SMX^r was found ($r^2 = 0.8251$, $P < 0.05$) (Table 3). This suggests that other selective pressures such as SMT and other chemicals were suspected to cause SMX^r. In particular, cross resistance to SMT could prompt the development of SMX^r bacteria (Iliades *et al.*, 2005). Another possible reason for the high incidence of SMX^r bacteria may be due to the persistence of SMX^r bacteria in the aquatic environments even when the concentration of SMX was low or even if SMX was not present. This phenomenon has been observed by Enne *et al.* (2001) and Bean *et al.* (2005) in clinical cases. Their studies showed that the persistence of sulfonamide resistance in *E. coli* in the UK despite the prescribed national restriction. Although there was no selective pressure by corresponding drug, sulfonamide-resistant bacteria can remain stably for 5 or 10 years in UK (Enne *et al.*, 2001). It can be concluded that, in the present study area also, SMX^r bacteria may persist even if SMX was not used or decline by the degradation processes.

In summary, our study revealed the status of drug contamination and drug-resistant bacteria in VAC wastewater; however, one-to-one correlation between them is not evident.

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