Antibiotic resistance in bacteria from shrimp farming in mangrove areas

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Abstract

Shrimp farming is a sufficiently large and mature industry to have an effective range of antimicrobial agents for most bacterial diseases in shrimp culture. However, at present, there exists great concern over the widespread use of antibiotics in aquaculture, which may result in residue of antibiotics in water and mud, and subsequently, the development of antibiotic resistance in bacteria in the environment. There is limited understanding about the effect of antibiotic residues on bacteria resistance in shrimp farming environment. Therefore, a study was conducted to investigate bacterial resistance to Norfloxacin (NFXC), Oxolinic Acid (OXLA), Trimethoprim (TMP) and Sulfamethoxazole (SMX), which were found in four shrimp farming locations in mangrove areas in Vietnam. Findings indicate that there is a relatively high incidence of bacteria resistance to these antibiotics observed in most of the studied sites, particularly to antibiotics with concentration of 0.1 μg/ml. Yet the relation between concentration of antibiotic residues and incidence of antibiotic resistance is not clearly defined. Among individual antibiotics, the incidence of resistance to TMP and SMX was higher than the others. Identification of bacteria isolated from mud samples by DNA analyzer shows that *Bacillus* and *Vibrio* are predominant among bacteria resistant to the antibiotics. The result of the study also indicates that these antibiotics in media degraded more rapidly due to the presence of resistant bacteria.
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1. Introduction

In Vietnam, shrimp production in coastal areas has been developed rapidly. The number of shrimp culture areas to rear tiger shrimp (*Penaeus monodon*) has grown significantly over the past ten years in response

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to the increasing market demand. Shrimp diseases are among the biggest concerns in shrimp farming in Vietnam (Uyen, 2002). As far as intensive shrimp culture goes, a large amount of shrimp food and antibiotics have been used to increase production and to protect shrimp from diseases (EJF, 2003). Consequently, a large portion of feeds and antibiotics enters the water as wastes, causing water pollution (Le et al., 2003). Several studies demonstrated the presence of antibacterial residues in fish farms (Weston, 1996; Capone et al., 1996; Herwig et al., 1997). Recent studies have shown that many antibiotics persist in the sediment and in the aquatic environment for several months following administration (Björklund et al., 1991; Lunestad et al., 1995; Pouliquen and Le, 1996; Hirsch et al., 1999; Miranda and Zemelman, 2002). The residues of antibacterial agents may affect the sedimentary microbial community and introduce antibiotic resistance in the bacteria (Hektoen et al., 1995; Tendencia and De la Pena, 2002). McPhearson et al. (1991) observed that individual and multiple antibiotic resistance were associated with antimicrobial use. Researchers reported that bacteria were resistant to Oxolinic Acid (OXLA) and bacteria resistance to Trimethoprim (TMP) and Sulfamethoxazole (SMX) were common (Hansen et al., 1992; Nygaard et al., 1992; Holmoström et al., 2003). A study in Thailand indicated that the pattern of antibiotic use among the farms could cause the risk of the development of resistant bacteria strains (Holmoström et al., 2003). Little is known about the occurrence of antibiotic-resistant bacteria in marine sediments near fish farms (Shmidt et al., 2000; Tendencia and De la Pena, 2001).

Quite high concentrations of Norfloxacin (NFXC), OXLA, TMP, and SMX were found in sediments in mangrove-based shrimp farming areas in Vietnam (Munekage et al., 2002; Le and Munekage, 2004). This may cause bacteria in shrimp culture areas to become antibiotic resistant. Therefore, this study is aimed at providing an understanding of the resistance of bacteria to the mentioned antibiotics (TMP, SMX, NFXC and OXLA) in a shrimp farming environment. Antibiotic-resistant bacteria isolated from mud samples are to be identified. In addition, research is also conducted into antibiotic degradation with the presence of the antibiotic-resistant bacteria isolated from mud samples.

2. Materials and methods

2.1. Study sites

Four sites were selected in tiger shrimp farming regions located in mangrove areas of Thai Binh Province (TB), Nam Dinh Province (ND), Can Gio–Ho Chi Minh City (CG), and Ca Mau Province (CM) in Vietnam. These sites were also included in our previous survey on detection of residues of TMP, SMX, NFXC, and OXLA in water and mud (Munekage et al., 2002; Le and Munekage, 2004). The samples were taken from one pond in each of the studied sites during the rearing period of 2003. The average size of these ponds was 0.5 hectare and the depth of these ponds was about 1.4–1.6 m. Salinity ranged from 15–20 psu.

2.2. Sampling

Water temperature ranged from 25–28 °C during sampling time. Samples were collected in the middle of each pond, at the depth of 0–20 cm from the surface, using 100 ml sterile bottles (APHA, 1989). Mud samples were obtained at the upper layer (0–5 cm depth) using divers and stored in 100 ml sterile bottles. All of the samples were processed for bacterial counts and isolation within 24 h.

2.3. Bacterial counts

One gram of mud sample or 1 ml of water sample was homogenized in 10 ml seawater, which was sterilized in an autoclave at 121 °C for 15 min. The homogenized sample was diluted and serial 10-fold dilution up to 10^4 was prepared in autoclaved seawater. Portions (0.1 ml) from each dilution were plated onto the Marine Agar media (pH: 7.2) in triplicate. Plate counts of bacteria were determined on Marine Agar—MA (APHA, 1989); 15 g marine broth (Difco), 10 g glucose, 5 ml pepton and 1 g extract yeast (Tokyo, Japan), 1000 ml seawater, which was sterilized by autoclave at 121 °C for 15 min. Autoclaved seawater was used for dilution. Media sets each contained one of the four individual antibacterial agents with different equivalent concentration: TMP (0.1 μg/ml, 1 μg/ml and 10 μg/ml), SMX (0.1 μg/ml, 1 μg/ml and 10 μg/ml), NFXC

(0.1 µg/ml, 1 µg/ml and 10 µg/ml) and OXLA (0.1 µg/ml, 1 µg/ml and 10 µg/ml) were used respectively. Media sets without any antimicrobial agents were also prepared at the same time. Formed colonies were counted after 24–48 h incubation under aerobic condition at 25 °C. The fraction of resistant bacteria was given as the percentage of colony forming unit (CFU) on the plates containing antibacterial agents compared to the CFU on plates without the addition of the antibiotic agents.

2.4. Isolation of bacteria

Representative colonies, which grew on plates containing antibacterial agents with concentrations of 10 µg/ml and were isolated from mud samples, were chosen. These isolates were cultured in Nutrient Liquid Media according to APHA (1989) with small modification in incubation at 25 °C. Then, colonies growing on these liquid media, which were shaken at a speed of 120 rpm, were used for identification and experimentation on antibiotics degradation in artificial seawater after 72 h.

2.5. Bacteria identification

2.5.1. DNA preparation

To prepare genomic DNA from cultured bacteria strains, the bacteria from 100 ml of Nutrient Liquid Media after 72 h of incubation were centrifuged at 8000 $\times g$ for 10 min. Then the pellets were used for DNA isolation with AquaPure Genomic DNA Kit (Bio-Rad, USA) according to the manufacturer's instructions. The yield of genomic DNA by agarose gel electrophoresis was confirmed.

2.5.2. Polymerase chain reactions

DNA fragments containing 16S ribosomal RNA gene were amplified by polymerase chain reaction (PCR) with the primers (E1; 5′-GAGTTTGATC-CTGGCTCAG-3′, and E2; 5′-TTTGCGCTCGT-TGCGGGACT-3′). Each reaction mixture contained 10 mM Tris–HCl (pH 8.3), 50 mM KCl, 1.5 mM MgCl$_2$, 0.4 mM each dNTP, 0.2 µM each primer, 2.5 units of Ex Taq DNA polymerase (Takara, Japan), and 100 ng of the template DNA. The PCR conditions were as follows: 94 °C for 30 s, 55 °C for 30 s, and 72 °C for 1 min (repeat 30 cycles), then

kept at 72 °C for 7 min as final extension. DNA fragments containing the PCR mixtures were analyzed by agarose gel electrophoresis, and DNA fragments (about 1 kb) were recovered and extracted from the gel with QIAquick Gel Extraction Kit (Qiagen GmbH, Germany).

2.5.3. DNA sequencing and bacterial identification

The recovered DNA fragments were analyzed by ABI3100-Avant Genetic Analyzer (Applied Biosystems, USA), according to the manufacturer's instructions. For identification of bacteria, obtained DNA sequences were submitted to a DNA similarity search program, BLAST (http://blast.genome.ad.jp).

2.6. Antibiotics analysis

TMP, SMX, NFXC, and OXLA were analyzed using high performance liquid chromatography (HPLC), according to Le and Munekage, (2004).

2.7. Degradation of antibiotics in seawater under laboratory conditions

In experiment on degradation of NFXC, OXLA, TMP, and SMX, solutions composed of 300 ml autoclaved artificial seawater, pepton (1.5 g), glucose (3 g), yeast extract (0.3 g), and one individual antibiotics agent (NFXC, OXLA, TMP, or SMX) with different equivalent concentrations (0.1 µg/ml, 1 µg/ml and 10 µg/ml) were prepared. After 72 h incubation, 0.5 ml of Nutrient Liquid Media containing bacteria from each location was taken and mixed to have 2 ml solutions containing resistant bacteria to NFXC, OXLA, TMP or SMX. These mixtures of resistant bacteria were added into the above prepared artificial seawater containing the studied antibiotics respectively. Non-bacterial solution was also prepared as a control at the same time. These solutions were shaken at 120 rpm and kept at 25 °C in the incubator. During the experimental period, 2 or 5 ml solutions were taken from each solution to determine amounts of antibiotics in the solution.

The data was analyzed for first-order kinetics by fitting to the

$$C_t = C_i \exp(-\alpha t), \tag{1}$$

where C_i is initial antibiotic concentration, α—degradation coefficient constant and t—experiment time. This equation can be converted into

$$t = -\ln(C_t/C_i)/\alpha \qquad (2)$$

Using Eqs. (1) and (2), degradation coefficient α and half-life of antibiotics ($T_{1/2}$) at the time when $C_t=1/2C_i$ will be calculated.

3. Results and discussion

3.1. Total and resistant bacterial viable count

3.1.1. Water samples

Antibiotic residues found in water samples taken in ponds in the studied locations are presented in Fig. 1. The figures indicate that residues of NFXC, OXLA, TMP, and SMX were found in the all samples. As shown in Fig. 1, the residues of all studied antibiotics in water samples taken in CM were higher than those in the other locations. Among them, NFXC and TMP residues were very high with values of 1.64 and 1.69 ppm. SMX concentrations however were not much different across the locations, generally within a range of 0.97–1.27 ppm.

Table 1 presents the analyzed results of the total bacterial count and antibiotic resistance in bacteria from selected water samples. The total bacterial count was found the highest in CM and the lowest in ND.

Analyses of the water samples reveal that bacteria were found to be resistant to NFXC, OXLA, TMP and SMX to different extents. The incidence of bacteria resistance to these antibiotics was reduced when the concentration of such antibiotics in the agar media increased (from 0.1–10 μg/ml). The highest incidence of bacteria resistance to NFXC, OXLA, TMP and SMX with concentration 0.1 μg/ml was identified in CM, which respectively measured at 52.11%, 42.33%, 67.97% and 67.22%, among the other ponds in TB, ND and CG. Meanwhile this incidence was observed at the lowest in CG (NFXC—22.47%, OXLA—28.19%, TMP—57.20%, and SMX—54.11%). Among individual antibiotics, the incidence of bacteria resistance to TMP and SMX was higher than that to NFXC and OXLA. With respect to CM samples, the incidence of bacteria resistance to NFXC, TMP and SMX as observed in media with higher concentration (1 and 10 μg/ml) was the highest compared to other studied locations.

The findings show that the highest incidence of bacteria resistance to NFXC, TMP and SMX with concentrations of 0.1, 1 and 10 μg/ml and OXLA with concentration of 0.1 μg/ml was observed in CM where the concentration of antibiotic residues was also found to be the highest in comparison to other locations. The lowest incidence of bacteria resistance to NFXC and OXLA was found in CG where the concentration of these antibiotics in water samples was at the lowest. It can be noted that the incidence of bacteria resistance
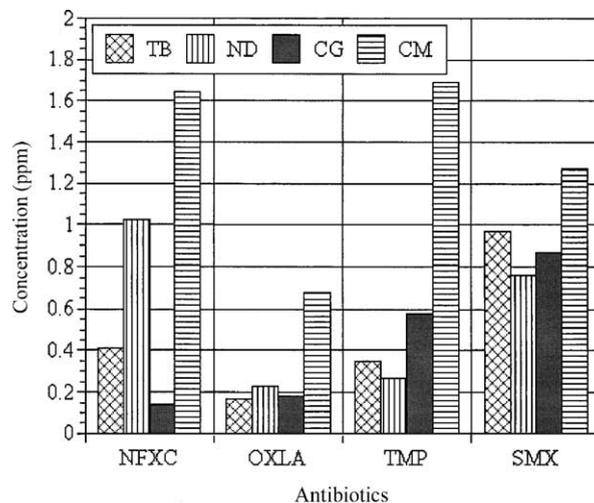

Fig. 1. Antibiotic residues in water samples.

Table 1
Total bacterial count on Marine Agar medium and percentage recovery of bacteria resistant to TMP, SMX, NFXC and OXLA from shrimp farming

Sample	Total bacterial count (cfu/ml for water and cfu/g for mud samples)	Bacteria resistant to NFXC (%)			Bacteria resistant to OXLA (%)			Bacteria resistant to TMP (%)			Bacteria resistant to SMX (%)		
		0.1 (µg/ml)	1 (µg/ml)	10 (µg/ml)	0.1 (µg/ml)	1 (µg/ml)	10 (µg/ml)	0.1 (µg/ml)	1 (µg/ml)	10 (µg/ml)	0.1 (µg/ml)	1 (µg/ml)	10 (µg/ml)
Water-TB	59×10^5	27.98	16.40	6.01	36.72	21.30	11.24	57.69	33.92	14.35	54.22	27.07	11.09
Water-ND	32×10^5	43.21	20.41	7.15	38.94	19.72	9.23	61.13	37.97	18.11	66.04	30.77	16.14
Water-CG	41.5×10^5	22.47	8.03	4.22	28.19	13.07	no data	57.20	30.13	14.51	54.11	27.77	12.14
Water-CM	71×10^5	52.11	20.92	8.92	42.33	15.34	5.88	67.97	39.01	18.53	67.22	30.22	21.97
Mud-TB	124×10^5	44.92	29.17	13.18	51.11	28.92	17.27	69.87	49.17	26.72	75.08	44.96	20.93
Mud-ND	103.4×10^5	60.91	36.19	19.21	59.71	39.47	21.41	77.04	no data	28.49	71.67	47.91	22.47
Mud-CG	98.7×10^5	38.24	no data	9.04	39.87	27.11	11.28	79.23	37.27	21.76	71.23	41.19	17.20
Mud-CM	153×10^5	57.67	28.91	17.15	51.76	28.49	no data	78.17	42.14	22.07	69.78	36.24	26.15

to antibiotics might be higher in the samples in which residues of these antibiotics was higher. However, for other samples, higher incidence of bacteria resistance to antibiotics could be found in the ponds, where antibiotics concentration was lower. It is suggested that the incidence of bacteria resistance to these antibiotics in shrimp pond water can also be influenced by other factors beside the antibiotic residues.

3.1.2. Mud samples

It can be seen from Fig. 2 that there is little difference in the NFXC concentrations in the mud samples in TB, ND and CG. The concentration of NFXC in CM was found much lower than that of the

Fig. 2. Antibiotic residues in mud samples.

other antibiotics in the other three locations. The OXLA concentrations in TB, ND and CM were found to be lower than 4.5 ppm but OXLA concentration in CG reached 11.23 ppm. Meanwhile, TMP concentration was quite high with a peak of 18.21 ppm in the mud sample taken in CM. In addition, the SMX concentrations in the studied locations varied between 6.87 and 12.56 ppm.

The incidence of bacteria resistance to NFXC, OXLA, TMP and SMX in mud samples is shown in Table 1. In general, the incidences of bacteria resistance to these antibiotics in mud samples were higher than those in water samples taken in the same location. Among the four locations, with concentration of 0.1 µg/ml, the incidence of bacteria resistance to NFXC was the highest in CM (60.91%); while the highest bacteria resistance to OXLA, was observed in ND at 59.71%. With respect to the media with higher concentration of antibiotics (1 and 10 µg/ml), the highest incidence of bacteria resistance to OXLA and NFXC was observed in ND.

It can be mentioned that the incidence of bacteria resistance to NFXC and OXLA was found to be highest in ND when NFXC concentration in mud was also highest in ND, but OXLA concentration was highest in CG. Although NFXC and OXLA concentration in mud samples in CG was quite low (1.85 and 1.23 ppm, respectively) the incidence of bacteria resistance to these antibiotics was high, which was 38.24% to NFXC 0.1 µg/ml and 39.87% to OXLA 0.1 µg/ml. Hansen et al. (1992) reported that the incidence of bacteria resistance to OXLA 10 µg/ml in artificial
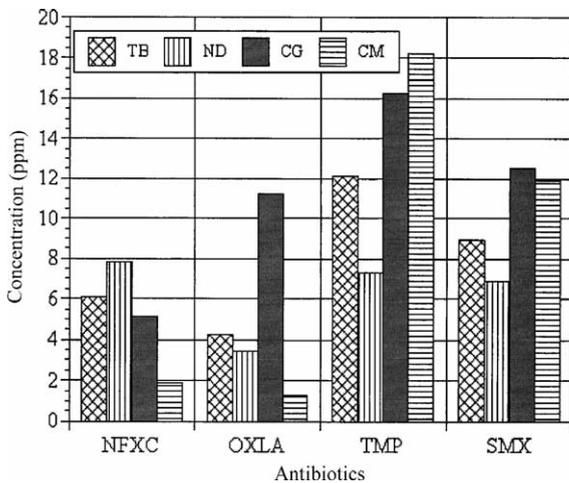
sediment increased to 20.3% and then decreased to 1.3% when the initial OXLA concentration was at 100 ppm and reduced to >40 ppm. Yet the incidence of bacteria resistance in Nygaard's experiments in sediment with initial OXLA concentration of 10 ppm rose from 7% to 20% when OXLA concentration decreased from 5 to 1 ppm. Thus it can be seen that the incidence of bacteria resistance to OXLA 10 μg/ml in our study (11.28–21.41%) is much higher than that in Hansen's experiment and similar to Nygaard's experiment while the amount of OXLA residues in our mud samples was much lower (3.45–11.23 ppm). However, the use of different media to cultivate bacteria may affect the results.

It can be noted that there is insignificant difference in bacteria resistance to TMP and SMX in mud samples among the locations even though TMP and SMX concentrations in mud were quite different; ND and CM are examples in case. Table 1 indicates that the incidences of bacteria resistance to TMP and SMX in mud samples were also higher than those to NFXC and OXLA. In a study taken by Elisabetta et al. (2003), all Gram-negative strains were totally resistant to SMX in both fish-farm and control stations. The findings of that study suggests that high incidence of antibiotic resistance in bacteria isolated from shrimp pond mud confirmed the widespread antimicrobial tolerance within aquatic bacteria, which is in agreement with the conclusions given in previous studies (Elisabetta et al., 2003). In addition, the high incidence of bacteria resistance to the studied antibiotics in all samples suggests that residues of antibiotics may favor the growth of resistant bacteria. Apart from visible factors such as antibiotics residues in the environment, the history of antibiotics use in the studied shrimp ponds can affect the level of bacteria resistance to antibiotics. According to some researchers, the frequency of antibiotic resistance is directly related to the volume of antibiotic used (Anderson and Levin, 1999). Tendencia and De la Pena (2001, 2002) also pointed out that when antimicrobial agents including OXLA are used more often in an environment, the higher will be the occurrence of resistant micro-organisms in that site. However, Weston (1996) stated that antibacterial resistance does not always respond in a predictable fashion correlating with the amount of drug used or with the concentration in the environment. The study

results on residues of NFXC, OXLA, TMP and SMX and their resistance in bacteria in mud in shrimp ponds indicate high risk of bacterial resistance and the impact on bacterial communities.

Generally, antibiotics concentration in most water and mud samples in this study was in the range of that in samples collected in 2002 in the studied locations, but it was much lower than the highest value found in 2002 (Le and Munekage, 2004). Thus, the control of antibiotic use and antibiotic resistance in water as well as in mud in shrimp culture areas are necessary.

3.2. Bacteria identification

Species of bacteria that grew in media containing NFXC, OXLA, TMP and SMX with the concentration of 10 μg/ml were identified in mud samples and determined by DNA analyzer. All the identified bacteria belong to Gram-negative and Gram-positive groups and are presented in Table 2. The bacteria identification shows that *Bacillus* strains were found in all isolates from NFXC-containing media in all studied locations. These strains were also found in isolates from OXLA-containing media in TB, ND and CM. In relation to TMP and SMX, *Bacillius* strains were identified in TB and ND, but not in CG and CM. In particular, *Bacillus atrophaeus* was resistant to NFXC, OXLA and SMX as revealed in samples taken in TB. In ND samples, *Bacillus anthracis* was found resistant to NFXC, OXLA and SMX. According to Elisabetta et al. (2003), the incidence of multiple resistance patterns in bacteria isolates was greater in impacted sediments. The presence of *Bacillus* strains producing antimicrobial compounds may be related to the high level of drug resistance. Therefore, the presence of *Bacillus* strains in most of our samples contribute to the high incidence of bacteria resistance to the studied antibiotics. *Vibrio* strains are another common species of bacteria in most of the samples. According to Tendencia and De la Pena (2002), *Vibrio* levels of the pond samples were higher from ponds where no antimicrobials had been used. But in our study, *Vibrio agarivorans* was resistant to all studied antibiotics in most samples except to NFXC in ND. In addition, other *Vibrio* strains were found to be resistant to studied antibiotics in CM.

Table 2
Bacterial species isolated from mud samples

Location	From medium that contained 10 μg/ml NFXC	From medium that contained 10 μg/ml OXLA	From medium that contained 10 μg/ml TMP	From medium that contained 10 μg/ml SMX
Thai Binh	*Bacillus atrophaeus*	*Bacillus atrophaeus*	*Escherichia coli*	*Escherichia coli*
	Vibrio agarivorans	*Vibrio agarivorans*	*Vibrio agarivorans*	*Bacillus atrophaeus*
	Bacillus mojavensis	*Bacillus subtilis*	*Bacillus mojavensis*	*Vibrio agarivorans*
		Bacillus sp.		*Bacillus* sp.
Nam Dinh	*Bacillus cereus*	*Escherichia coli*	*Escherichia coli*	*Escherichia coli*
	Bacillus anthracis	*Bacillus atrophaeus*	*Bacillus anthracis*	*Vibrio agarivorans*
	Bacillus atrophaeus	*Vibrio agarivorans*	*Vibrio agarivorans*	*Bacillus anthracis*
		Bacillus anthracis		
Can Gio	*Vibrio agarivoran*	*Vibrio agarivorans*	*Vibrio agarivorans*	*Vibrio agarivorans*
	Bacillus atrophaeus	*Escherichia coli*	*Thiobaca trueperi*	*Oceanospirillum* sp.
	Pseudomonas sp.			
Ca Mau	*Bacillus atrophaeus*	*Vibrio agarivorans*	*Vibrio agarivorans*	*Vibrio agarivorans*
	Vibrio agarivorans	*Bacillus atrophaeus*	*Escherichia coli*	*Listonella anguillarum*
	Vibrio sp.	*Escherichia coli*	*Listonella anguillarum*	*Vibrio* sp.
		Vibrio sp.	*Vibrio* sp.	

The results reveal that *Pseudomonas* was resistant to NFXC in only one sample in CG. *E. coli* was resistant to OXLA, TMP, and XMX in some locations but not resistant to NFXC. *Thiobaca trueperi*, *Listonella anguillarum*, *Listonella anguillarum*, and *Oceanospirillum* sp. were found to be resistant to TMP and SMX. Multi-antibiotic resistance has been observed in bacteria from aquaculture environments, which is often associated with the use of some drugs (McPhearson et al., 1991; Shmidt et al., 2000). In addition, Nygaard et al. (1992) reported that the exposure to OXLA initiates resistance to other drugs; and according to Hansen et al. (1992), bacteria exposed to OXLA developed cross-resistance towards flumequine and OTC. Therefore, the high bacterial resistant level to OXLA in our study suggests the possibility of resistance/or cross-resistance to other drugs in studied locations. It is known that TMP, SMX, NFXC, and OXLA have been used to control bacteria such as *Vibrio* and *Pseudomonas* spread on a wide scale. However, in this study, some of these bacterial strains were found to be resistant to the used antibiotics; this may be possible for disease breakdown in the studied location although not all *Vibrio* and *Pseudomonas* can cause diseases in shrimp. The study results suggest that further investigations into the ability of these antibiotics to initiate resistance to other drugs is necessary.

3.3. Antibiotics degradation in artificial seawater under laboratory conditions

The results of antibiotic concentrations in artificial seawater over 240 h are presented in Fig. 3, in which the concentrations of NFXC, OXLA, TMP, and SMX in the samples decreased more rapidly compared to those in the control. The exponential plots in Fig. 3 show a good relationship indicating first-order kinetics ($r^2 \geq 0.9583$). Therefore, Eqs. (1) and (2) were applied to calculate the degradation coefficient α and half-life of antibiotics ($T_{1/2}$).

The degradation coefficient α presented in Fig. 4 shows that in control solutions containing initial antibiotics concentration of 1 and 10 μg/ml, degradation coefficient was in the order: SMX>TMP>OXLA>NFXC, but it was TMP>SMX>OXLA>NFXC in solutions containing 0.1 μg/ml (initial concentration) of these antibiotics. In both control and samples, the degradation coefficient of studied antibiotics decreased when the initial antibiotics concentration increased.

With respect to seawater samples containing bacteria, SMX had the highest coefficient of degradation compared to NFXC, OXLA, and TMP with all initial antibiotic concentrations. The degradation coefficients of NFXC and TMP were similar in solutions with initial antibiotic concentrations of 0.1
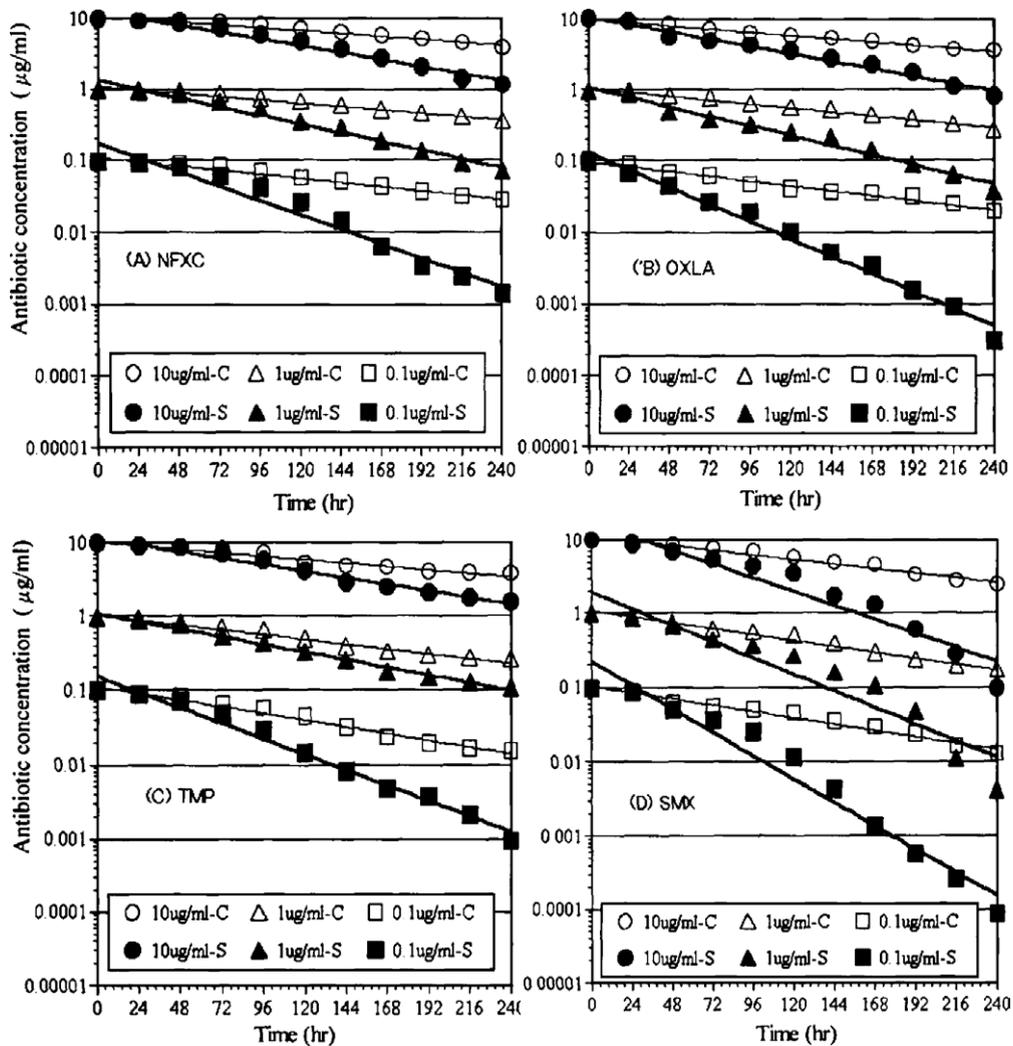
Fig. 3. Degradation of antibiotics concentration in artificial seawater.

and 10 μg/ml. In the solutions with initial concentration of 10 μg/ml, the degradation coefficient of OXLA is similar to those of TMP and NFXC but quite higher than those of TMP and NFXC in solutions with initial concentration of 0.1 μg/ml. Generally, the order of degradation coefficient of these antibiotics in samples containing bacteria was as follows:

– In solution with initial concentration of 0.1 μg/ml: SMX>OXLA>TMP>NFXC
– In solution with initial concentration of 1 and 10 μg/ml: SMX>OXLA>NFXC>TMP

Fig. 4 indicates that the degradation coefficient of studied antibiotics in sample solutions ranged from 2.1 to 3.7 times higher than that in the control.

The data obtained from this experiment were also used to calculate half-lives $(T_{1/2})$ of the studied antibiotics in artificial seawater under laboratory conditions at three different initial concentrations (0.1, 1 and 10 μg/ml) as presented in Table 3.

Table 3 indicates that among the individual antibiotics, the half-life of NFXC in both samples and control at three different concentrations reached the highest values of 69.2, 86.6, 102.6 h and 145.3, 176.5, 198.4 h, respectively. In lower initial anti-
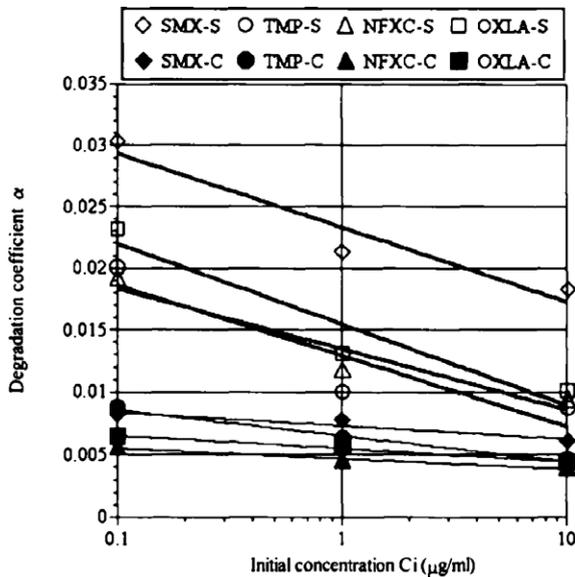
Fig. 4. Degradation coefficient of antibiotics in artificial seawater.

biotics concentrations (0.1 and 1 ppm), OXLA degraded more rapidly in samples ($T_{1/2}$=45.7 and 62.9 days) compared to other antibiotics. SMX half-life was the lowest among individual studied antibiotics in the solution with initial antibiotics concentration of 10 ppm.

The results obtained from the experiment on antibiotics degradation indicate that half-life of all studied antibiotics in the samples containing resistant bacteria were lower than that in the control. Among the individual antibiotics, SMX was the most rapidly degraded in samples with all initial antibiotic concentration levels. It is mentioned that the degradation coefficient of SMX was higher than

that of other antibiotics by far. In seawater with an initial antibiotic concentration of 10 μg/ml, NFXC and TMP were slowly degraded, but rapidly degraded in seawater with 0.1 μg/ml. Meanwhile, OXLA was also slowly degraded with a level so close to that of NFXC in initial concentration at 10 μg/ml level, but more degraded in initial concentration at the 0.1 μg/ml level. The finding from this experiment is quite similar to the suggestion by Hektoen et al. (1995) regarding the microbiological degradation of TMP, SMX, and OXLA by the sediment bacteria. This also may help explain why the antibiotic residue level was low but the incidence of bacteria resistance remained high. The study results show that the degradation order of studied antibiotics in samples was not like those in the control condition; it suggests that the presence of bacteria in solution changed the order of degradation coefficient in the sample solution due to differing bacteria capacity to degrade antibiotics. Generally, in seawater with initial antibiotic concentration of 0.1 μg/ml, these resistant bacteria were found to be the most effective in degrading antibiotics TMP, SMX, and OXLA.

Because of limitations, there are some initial findings found in this study and further study on antibacterial degradation by bacteria needs to be carried out.

4. Conclusion remarks

For the purpose of this study, antibiotic residues and incidence of bacteria resistance to NFXC,

Table 3

Half-lives ($T_{1/2}$) in hours (h) of the studied antibiotics in artificial seawater under laboratory conditions at three different initial concentrations

Antibiotics		Initial antibiotic concentration: 0.1 μg/ml		Initial antibiotic concentration: 1 μg/ml		Initial antibiotic concentration: 10 μg/ml	
		$T_{1/2}$	R^2	$T_{1/2}$	R^2	$T_{1/2}$	R^2
Norfoxacin	Sample	69.2	0.9583	86.6	0.9729	102.6	0.9645
	Control	145.3	0.9814	176.5	0.9781	198.4	0.9616
Oxolinic acid	Sample	45.7	0.9836	62.9	0.9807	76.6	0.9774
	Control	102.4	0.9792	146.6	0.984	166.2	0.993
Trimethoprim	Sample	57.8	0.9811	81.9	0.9897	99.8	0.9755
	Control	98.9	0.9772	123.6	0.9787	160.9	0.9521
Sulfamethoxazole	Sample	54.0	0.9583	69.5	0.8977	73.5	0.9156
	Control	92.2	0.9771	104.0	0.9844	134.4	0.9705

OXLA, TMP and SMX were observed in shrimp ponds in different mangrove areas in Vietnam. It is revealed that antibiotic use in shrimp ponds leads to residues of antibiotics in water and mud; and consequently, causes bacteria resistance to the antibiotics. Several bacterial strains such as *Bacillus* and *Vibrio* strains were found in mud samples and were resistant to the studied antibiotics in most of the study locations. With the presence of resistant bacteria to NFXC, OXLA, TMP, and SMX in artificial seawater which contained these antibiotics respectively, antibiotic concentration was decreased more rapidly than in artificial seawater without the bacteria. The findings of antibiotic-resistant bacteria also implies an urgent need for a monitoring system of antibacterial drugs that are being used in shrimp farming.

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