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Antimicrobial Susceptibility of Indicator Bacteria Isolated from Chickens in Southeast Asian Countries (Vietnam, Indonesia and Thailand)

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ABSTRACT. To determine the prevalence of indicator bacteria resistant to antimicrobials among poultry in three Southeast Asian countries (Vietnam, Indonesia and Thailand), we examined the antimicrobial susceptibilities of commensal bacteria isolated from chickens. In total, 125, 117 and 180 isolates of *Escherichia coli*, *Enterococcus faecalis* and *Enterococcus faecium*, respectively, were used to test for antimicrobial susceptibility. Bacterial resistance to antimicrobial treatment was most frequently observed with oxytetracycline with a prevalence of 73.6% (*E. coli*), 69.2% (*E. faecalis*) and 92.2% (*E. faecium*). Resistance to fluoroquinolones, which are critically important medicines, was also frequently observed in *E. coli* (48.8%), *E. faecalis* (17.9%) and *E. faecium* (82.8%). The prevalence of indicator bacteria resistant to most of the antimicrobials tested in these countries was higher than those for developed countries. The factors underlying antimicrobial resistance may include inappropriate and/or excessive use of antimicrobials. These results highlight the need for monitoring the emergence and prevalence of antimicrobial resistance in developing countries.
KEY WORDS: antimicrobial resistance, developing countries, indicator bacteria, monitoring, Southeast Asia.

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The emergence and spread of antimicrobial resistance is a global concern for both human and veterinary medicine. Swan *et al*. first highlighted the threat of transmission of antimicrobial resistance from food-producing animals to humans [26]. The proposed mechanism for the transmission of antimicrobial resistance was the overuse of antimicrobials during veterinary care. To address these issues, the World Health Organization (WHO) and other international organizations initiated programs to monitor antimicrobial resistance in zoonotic bacteria, animal pathogens and indicator bacteria derived from food-producing animals [9, 13].

Many developed countries, such as Japan, the United States and Denmark, have national monitoring programs for assessing bacterial susceptibility to antimicrobials among enteric bacteria isolated from seemingly healthy animals [8, 21, 27]. The results from these programs revealed that antimicrobial resistance is highly prevalent in food-producing animals. In response to these monitoring data, the governments of some developed countries have adopted a set of control measures [1, 14, 18].

In developing countries, very little data have been published regarding the occurrence of antimicrobial-resistant bacteria in food-producing animals. Some studies have shown that antimicrobial use in food-producing animals was unregulated and/or that antimicrobials are inappropriately used, resulting in a widespread increase in antimicrobial-resistant bacteria in Southeast Asian developing countries [11, 28, 29]. Due to rapid globalization, increasing antimicrobial resistance in developing countries is now of concern for other nations.

In many Southeast Asian developing countries, such as Vietnam, Indonesia and Thailand, the flourishing poultry industries export live chicken and chicken meat all over the world. Previous studies have shown that the prevalence of bacteria resistant to antimicrobials is higher in chicken than in cattle and pigs, since all the animals in the affected poultry flocks are preferably treated with antimicrobials [8, 11, 19, 21]. Therefore, additional surveys regarding the extent of antimicrobial resistance and antimicrobial use in food-producing animals, especially in chickens, in Southeast Asian countries are required.

In this study, we examined chicken fecal samples in accordance with a Japanese national monitoring program [16, 19] to determine the antimicrobial resistance of indicator bacteria (*Escherichia coli* and *Enterococcus* spp.) in three Southeast Asian countries, namely, Vietnam, Indonesia and Thailand. This study was conducted as a pilot study. *E. coli* and *Enterococcus* spp. are useful indicator bacteria for estimating the usage of antimicrobials in chickens and comparing the prevalence of bacteria resistant to antimicrobials

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Table 1. Antimicrobial susceptibility of *E. coli* isolates from chickens obtained from Southeast Asian countries

Antimicrobial	Break point (mg/*l*)[a)]	MIC range (mg/*l*)	MIC_{50} (mg/*l*)	MIC_{90} (mg/*l*)	% of antimicrobial resistance		
					Vietnam (n=47)	Indonesia (n=78)	Total (n=125)
Ampicillin	32	1–>512	256	>512	83.0[b)]	43.6	58.4
Cefazolin	8	0.25–512	2	8	4.3	5.1	4.8
Cefpodoxime	8	<0.125–128	0.25	1	4.3	5.1	4.8
Kanamycin	64	2–>512	2	>512	48.9[b)]	16.7	28.8
Gentamicin	16	0.3–>512	1	32	44.7[b)]	0	16.8
Dihydrostreptomycin	32	1–>512	8	512	82.9[b)]	24.4	46.4
Oxytetracycline	16	0.5–>512	128	512	93.6[b)]	61.5	73.6
Chloramphenicol	32	0.25–>512	8	256	51.1[b)]	25.6	35.2
Nalidixic acid	32	2–>512	128	>512	80.1[b)]	42.3	56.8
Enrofloxacin	2	<0.125–64	1	64	70.2[b)]	35.9	48.8

MIC: Minimal inhibitory concentration. a) The value was the CLSI break point, b) The percentage of antimicrobial resistance in Vietnam was significantly higher than that in Indonesia ($P<0.05$).

within and between developed countries [5, 16, 19, 20].

MATERIALS AND METHODS

Sample collection: Chicken fecal samples were collected from Vietnam, Indonesia and Thailand by local collaborators. All chickens were later utilized as poultry food products. The countries chosen in this study are areas of major chicken production in the region and are considered representative of chicken farming in other Southeast Asian countries. One hundred fecal samples were collected from a poultry slaughterhouse (suburbs of Hanoi) in Vietnam during September 2007. Fecal samples were obtained from chickens that had been brought from 23 farms located in 19 provinces (Bac Giang, Bac Ninh, Cao Bang, Ha Nam, Ha Noi, Ha Tay, Hai Duong, Hai Phong, Hung Yen, Long Son, Nam Dinh, Ninh Binh, Phu Tho, Quang Ninh, Thai Binh, Tahi Nguyen, Thanh Hoa, Tuyen Quang and Vinh Phuc) (5 samples/province; 10 samples were obtained from Ha Noi). From Indonesia, a total of 144 fecal samples were collected at seven farms from seven different provinces (North Sumatra, West Sumatra, Lampung, Yogyakarta, South Kalimantan, Bali and South Sulawesi) (20 to 22 samples/province) during January and February 2007. From Thailand, a total of 100 fecal samples were collected from three farms located in three provinces (Bangkok, Burirum and Surin) (43 samples/Bangkok, 37 samples/Burirum and 20 samples/Surin) during January 2006. All samples were placed in sterile plastic tubes and stored at −20°C or −80°C until use.

Bacterial isolation: To isolate *E. coli,* chicken fecal samples were spread onto desoxycholate-hydrogen sulfate-lactose agar (Nissui Pharmaceutical, Tokyo, Japan) and incubated at 37°C overnight. For individual samples, a maximum of 2 colonies were identified as *E. coli* on the basis of colony morphology and then selected for further analysis. To confirm that these colonies were *E. coli*, the selected colonies were then processed for API 20E testing (Sysmex, Kobe, Japan). Samples from Thailand were not used for *E. coli* isolation.

To isolate enterococci, chicken fecal samples were spread onto Enterococcosel (ECS) agar (Becton Dickinson, Franklin Lakes, NJ, U.S.A.), incubated at 37°C for 48–72 hr and enriched using ECS broth. After incubation at 37°C for 16 hr, enriched cultures were streaked onto ECS agar. For individual samples, we selected a maximum of two colonies identified as enterococci on the basis of colony morphology. The colonies were applied to the API 20 STREP system (Sysmex) for bacterial species identification. DNA was extracted from the colonies using a commercial kit (InstaGeneMatrix, BioRad, Tokyo, Japan) and following the manufacturer's instructions. To distinguish between *E. faecalis* and *E. faecium*, we performed multiplex PCR [12].

Antimicrobial susceptibility tests: We determined minimal inhibitory concentrations (MICs) using the agar dilution method according to guidelines set by the Clinical Laboratory Standards Institute (CLSI) [6]. We tested the following antimicrobials on *E. coli*: ampicillin, cefazolin, kanamycin, gentamicin, dihydrostreptomycin, oxytetracycline, chloramphenicol, nalidixic acid, enrofloxacin (all obtained from Sigma-Aldrich, St. Louis, MO, U.S.A.) and cefpodoxime (Daiichi-Sankyo, Tokyo, Japan). On *E. faecalis* and *E. faecium*, we tested the following antimicrobial agents: ampicillin, kanamycin, gentamicin, dihydrostreptomycin, erythromycin, lincomycin, oxytetracycline, vancomycin, chloramphenicol and enrofloxacin (all obtained from Sigma-Aldrich). The breakpoints were in accordance with CLSI guidelines [6]. The breakpoints for kanamycin, gentamicin and enrofloxacin for *E. faecalis* and gentamicin for *E. faecium* have not been defined by the CLSI, and therefore, we used the guidelines set by Kojima *et al*. [18]. In this study, we defined the breakpoint of kanamycin and enrofloxacin for *E. faecium* by taking into consideration the midpoint between the peaks of each MIC distribution. *E. coli* ATCC25922, *Staphylococcus aureus* ATCC29213, *E. faecalis* ATCC29212 and *Pseudomonas aeruginosa* ATCC27853 were used as quality control strains. *Enterococcus* isolates that exhibited low susceptibility to vancomycin (MIC=8 mg/*l*) were selected for further tests.

Detection of vancomycin-resistance genes: The vancomycin-resistance genes, *vanA, vanB, vanC1, vanC2/3, vanD*

Table 2. Multidrug resistant phenotypes of *E. coli* isolates from chickens obtained from Southeast Asian countries

No. exhibiting antimicrobial resistance	MDR patterns	No. of isolates		
		Vietnam (n=47)	Indonesia (n=78)	Total (n=125)
10	ACCdKGDOCpNE	2	0	2
8	AKGDOCpNE	6	0	6
7	AKGDONE	5	0	5
	AKDOCpNE	3	2	5
	AGDOCpNE	2	0	2
	AKGDOCpN	1	0	1
	ACKGDON	1	0	1
	ACCdKDNE	0	1	1
6	ADOCpNE	2	2	4
	AKDONE	3	0	3
	AGDOCpN	2	0	2
	AKDOCpN	1	0	1
	AKOCpNE	0	1	1
	KGDONE	0	1	1
5	AOCpNE	3	4	7
	ACCdKD	0	3	3
	AKONE	0	2	2
	ADONE	1	1	2
	KDONE	1	1	2
	ADOCpN	0	1	1
	AOCpNE	0	1	1
4	AONE	2	2	4
	ADON	3	0	3
	GDOCp	1	0	1
	DONE	1	0	1
	OCpNE	0	1	1
3	ONE	0	5	5
	DON	1	3	4
	DOC	0	3	3
	ADO	0	2	2
	AOCp	0	2	2
	AKN	0	1	1
	AKO	0	1	1
2	AO	0	3	3
	NE	0	3	3
	DO	0	2	2
	AD	1	0	1
	AG	1	0	1
	OCp	0	1	1

MDR: Multidrug Resistance, A: Ampicillin, C: Cefazolin, Cd: Cefpodoxime, K: Kanamycin, G: Gentamicin, D: Dihydrostreptomycin, O: Oxytetracycline, Cp: Chloramphenicol, N: Nalidixic acid, E: Enrofloxacin.

and *vanE*, were detected in the isolates that exhibited low susceptibility to vancomycin by performing PCR as previously described [12, 17].

Statistical analysis: *E. coli* resistance to the selected antimicrobial agents was compared between isolates from Vietnam and Indonesia using the χ^2 test. When a two-by-two table contained at least 1 expected value below 5, the Fisher's exact test was performed.

E. faecalis and *E. faecium* isolates were obtained from Vietnam, Indonesia and Thailand. The prevalence of antimicrobial resistant bacterial strains across each country was compared using multiple comparisons. A chi-square test was first performed for each antimicrobial agent to detect significant differences within a country. When the result was significant, a test for multiple comparisons of proportions [24] was performed using R (version 2.14.2) statistical software. If we did not identify any resistant isolates in 2 of the 3 countries, we performed a pairwise comparison between the country with the resistant isolate and the countries that did not have a resistant isolate using either the χ^2 test or Fisher's

Table 3. Antimicrobial susceptibility of *E. faecalis* isolates from chickens obtained from Southeast Asian countries

Antimicrobial	Break point (mg/*l*)	MIC range (mg/*l*)	MIC_{50} (mg/*l*)	MIC_{90} (mg/*l*)	% of antimicrobial resistance			
					Vietnam (n=22)	Indonesia (n=58)	Thailand (n=37)	Total (n=117)
Ampicillin	16[a)]	<0.125–8	1	2	0	0	0	0
Kanamycin	64[b)]	32–>512	64	>512	77.3[c), d)]	29.3	27	37.6
Gentamicin	512[b)]	4–>512	16	512	40.9[c), d)]	6.9	0	11.1
Dihydrostrepto-mycin	256[a)]	16–512	128	>512	95.4[c), d)]	41.4	40.5	51.3
Erythromycin	8[a)]	<0.125–>512	>512	>512	90.9[d)]	77.6[e)]	48.6	70.9
Lincomycin	128[a)]	32–>512	>512	>512	90.9[d)]	79.3[e)]	54.1	73.5
Oxytetracycline	16[a)]	1–512	256	>512	100[c), d)]	65.5	56.8	69.2
Vancomycin	32[a)]	0.5–8	2	4	0	0	0	0
Chloramphenicol	32[a)]	4–128	16	128	86.3[c), d)]	8.6	21.6	27.4
Enrofloxacin	16[b)]	0.25–128	1	64	36.4[d)]	19	5.4	17.9

MIC: Minimal inhibitory concentration. a) The value was the CLSI break point, b) The value was the JVARM breakpoint [13], c)–e) Superscripts indicate significantly higher percentage of antimicrobial resistance in Vietnam than in Indonesia (c), Vietnam than in Thailand (d), Indonesia than in Thailand (e) (P<0.05).

exact test. The criteria used to select between these tests are mentioned above (as described for *E. coli)*.

RESULTS

E. coli: In total, we identified 125 *E. coli* isolates and described any antimicrobial resistance for all agents tested (Table 1). The majority of *E. coli* isolates from Vietnam and Indonesia were resistant to oxytetracycline (73.6%), nalidixic acid (56.8%) and ampicillin (58.4%). The prevalence of *E. coli* isolates from Vietnam that were resistant to almost all antimicrobials, except cefazolin and cefpodoxime, was significantly higher than of those from Indonesia (P<0.05). The diversity of multidrug resistant phenotypes among the *E. coli* isolates is shown in Table 2. Forty-three (91.5%) of the 47 isolates obtained from Vietnam exhibited multidrug resistance, and the isolates were resistant to 2–10 different antimicrobials. Forty-nine (62.8%) out of 78 isolates obtained from Indonesia exhibited multidrug resistance, and each isolate was resistant to 2–7 antimicrobial agents.

E. faecalis: We identified 117 *E. faecalis* isolates in this study. The isolates exhibited antimicrobial resistance for 8 of the 10 agents tested (Table 3). *E. faecalis* resistance against lincomycin (73.5%), erythromycin (70.9%) and oxytetracycline (69.2%) was common in all 3 countries. We did not identify any isolates resistant to vancomycin. Three of the isolates derived from Indonesia exhibited low susceptibility to vancomycin (MIC=8 mg/*l*), but the isolates did not have vancomycin-resistance genes. The prevalence of *E. faecalis* isolates from Vietnam that were resistant to almost all antimicrobials tested was significantly higher than of those collected from other countries (P<0.05). The prevalence of *E. faecalis* isolates from Indonesia that were resistant to erythromycin and lincomycin was significantly higher than the prevalence of those from Thailand (P<0.05). The diversity of multidrug resistant phenotypes among *E. faecalis* isolates is shown in Table 4. Twenty-two (100%) out of 22 isolates obtained from Vietnam exhibited multidrug resistance and were found to be resistant to 2–8 different antimicrobials. Forty-nine (84.5%) out of 58 isolates obtained from Indonesia exhibited multidrug resistance and were found to be resistant to 2–6 different antimicrobials. Twenty-seven (73.0%) out of 37 isolates obtained from Thailand exhibited multidrug resistance and were determined to be resistant to 2–7 different antimicrobials.

E. faecium: In this study, 180 *E. faecium* isolates were identified. Antimicrobial resistance was found for nine of the 10 antimicrobials tested (Table 5). *E. faecium* isolate resistance to oxytetracycline (92.2%), lincomycin (83.9%), enrofloxacin (82.8%), erythromycin (79.4%) and kanamycin (62.2%) was common in all three countries. Isolates resistant to vancomycin were not found in all countries. One *E. faecium* isolate from Indonesia exhibited low susceptibility to vancomycin (MIC=8 mg/*l*), but the isolate did not contain vancomycin-resistance genes. The prevalence of *E. faecium* isolates from Vietnam that were resistant to almost all the antimicrobials tested was significantly higher than of those from Indonesia (P<0.05), except for kanamycin and vancomycin. The prevalence of *E. faecium* isolates from Vietnam that were resistant to ampicillin, gentamicin, dihydrostreptomycin and chloramphenicol was significantly higher than of those from Indonesia (P<0.05). The prevalence of *E. faecium* isolates from Thailand that were resistant to erythromycin, lincomycin, oxytetracycline and enrofloxacin was significantly higher than of those from Indonesia (P<0.05). The diversity of multidrug resistant phenotypes among the *E. faecium* isolates is shown in Table 6. Eighty-five (95.5%) out of 89 isolates obtained from Vietnam exhibited multidrug resistance and were resistant to 2–9 different antimicrobials. Fifty-three (91.4%) out of the 58 isolates obtained from Indonesia exhibited multidrug resistance and were resistant to 2–6 different antimicrobials. Thirty-three (100%) out of the 33 isolates obtained from Thailand exhibited multidrug resistance and were resistant to 2–7 different antimicrobials.

Table 4. Multidrug resistant phenotypes of *E. faecalis* isolates from chickens obtained from Southeast Asian countries

No. exhibiting antimicrobial resistance	MDR patterns	No. of isolates			
		Vietnam (n=22)	Indonesia (n=58)	Thailand (n=37)	Total (n=117)
8	KGDEmLOCpE	6	0	0	6
7	KGDEmLOCp	4	0	3	7
6	KGDEmLO	3	0	7	10
	KDEmLOCp	4	1	1	6
	KGEmLOCp	0	0	4	4
	KGDEmLE	0	3	0	3
	DEmLOCpE	0	3	0	3
5	KDEmLO	0	15	0	15
	KEmLOE	0	1	2	3
	DEmLOE	1	2	0	3
	KDEmLO	1	0	0	1
	DELOCp	0	1	0	1
	KDEmLE	0	1	0	1
	KDLOE	0	1	0	1
	KGEmLO	0	0	1	1
4	DEmLO	0	12	0	12
	KDEmL	0	2	0	2
	KEmLE	0	1	0	1
	GDEmL	1	0	0	1
	EmLOE	1	0	0	1
	DEmLE	0	1	0	1
3	KDL	0	0	1	1
	DOCp	1	0	0	1
2	KG	0	0	7	7
	KD	0	2	0	2
	DEm	0	1	0	1
	DL	0	1	0	1
	DE	0	1	0	1
	LO	0	0	1	1

MDR: Multidrug Resistance, A: Ampicillin, K: Kanamycin, G: Gentamicin, D: Dihydrostreptomycin, Em: Erythromycin, L: Lincomycin, O: Oxytetracycline, Cp: Chloramphenicol, E: Enrofloxacin.

Table 5. Antimicrobial susceptibility of *E. faecium* isolates from chickens obtained from Southeast Asian countries

Antimicrobial	Break point (mg/*l*)	MIC range (mg/*l*)	MIC_{50} (mg/*l*)	MIC_{90} (mg/*l*)	% of antimicrobial resistance			
					Vietnam (n=89)	Indonesia (n=58)	Thailand (n=33)	Total (n=180)
Ampicillin	16[a)]	<0.125–64	4	8	19.1[d),e)]	0	0	9.4
Kanamycin	64[b)]	8–>512	128	>512	50.6	69.0[f)]	81.8[h)]	62.2
Gentamicin	256[c)]	1–512	8	>512	27.0[d),e)]	0	3	13.9
Dihydrostreptomycin	256[a)]	16–>512	64	>512	61.8[d),e)]	29.3[g)]	15.2	42.8
Erythromycin	8[a)]	<0.125–>512	512	>512	91.0[d)]	51.7	97.0[i)]	79.4
Lincomycin	128[a)]	8–>512	512	>512	88.8[d)]	69	97.0 [i)]	83.9
Oxytetracycline	16[a)]	0.5–512	256	512	97.8[d)]	81	97.0 [i)]	92.2
Vancomycin	32[a)]	0.5–8	1	2	0	0	0	0
Chloramphenicol	32[a)]	2–128	8	16	10.1[d),e)]	0	0	5.0
Enrofloxacin	2[b)]	0.5–128	8	128	86.5[d)]	69	97.0 [i)]	82.8

MIC: Minimal inhibitory concentration. a) The value was the CLSI break point, b) The value was set as the midpoint between the peaks of each MIC distribution, c) The value was the JVARM breakpoint [13], d)–i) Superscripts indicate a significantly higher percentage of antimicrobial resistance in Vietnam than in Indonesia (d), Vietnam than in Thailand (e), Indonesia than in Vietnam (f), Indonesia than in Thailand (g), Thailand than in Vietnam (h), Thailand than in Vietnam (i) (P<0.05).

Table 6. Multidrug resistant phenotypes of *E. faecium* isolates from chickens obtained from Southeast Asian countries

No. exhibiting antimicrobial resistance	MDR patterns	No. of isolates			
		Vietnam (n=89)	Indonesia (n=58)	Thailand (n=33)	Total (n=180)
9	AKGDEmLOCpE	1	0	0	1
8	AKGDEmLOE	6	0	0	6
	KGDEmLOCpE	2	0	0	2
	AKDEmLOCpE	1	0	0	1
7	KGDEmLOE	13	0	0	13
	AGDEmLOE	3	0	0	3
	KGDEmLOCp	1	0	1	2
	KDEmLOCpE	1	0	0	1
6	KDEmLOE	7	10	3	20
	KGEmLOE	7	1	1	9
	ADEmLOE	5	0	0	5
	KDEmLOCp	1	0	0	1
5	KEmLOE	2	4	20	26
	DEmLOE	8	0	0	8
	KDEmLO	3	1	1	5
	KDLOE	0	2	0	2
	KDEmLE	0	1	0	1
4	EmLOE	15	4	6	25
	KEmLO	1	5	0	6
	KLOE	0	4	0	4
	KEmOE	2	0	0	2
	DEmLO	0	2	0	2
	KDLO	0	1	0	1
	KDLE	0	1	0	1
	DEmLE	0	1	0	1
3	LOE	1	4	0	5
	KLO	0	3	0	3
	KOE	0	3	0	3
	AOE	1	0	0	1
	KDO	1	0	0	1
	EmLE	1	0	0	1
	DEmL	0	1	0	1
2	OE	1	3	0	4
	KEm	0	0	1	1
	KE	0	1	0	1
	DO	1	0	0	1
	LO	0	1	0	1

MDR: Multidrug Resistance, A: Ampicillin, K: Kanamycin, G: Gentamicin, D: Dihydrostreptomycin, Em: Erythromycin, L: Lincomycin, O: Oxytetracycline, Cp: Chloramphenicol, E: Enrofloxacin.

DISCUSSION

In this study, *E. faecium* was more frequently isolated from chicken feces than *E. faecalis*, which is consistent with previous reports [3, 10] that suggested that *E. faecium* is the most predominant enterococcus in chicken feces. Resistance against enrofloxacin and ampicillin was more commonly observed in *E. faecium* than *E. faecalis*. In contrast, resistance to chloramphenicol was more commonly observed in *E. faecalis* than *E. faecium*. The same trend has been observed in several previous studies [4, 19]. These results suggest that the frequency of acquiring antimicrobial resistance differs according to the species.

The prevalence of *E. coli* and enterococci isolates resistant to antimicrobials, especially fluoroquinolone, was higher than in developed countries [5, 16, 18]. In this study, most *E. coli* and enterococci isolates exhibited multidrug resistance. In all three countries, *E. coli* and enterococci resistance to oxytetracycline was most common. Previous work has shown that a resistance of indicator bacteria against each class of antimicrobial is proportional to the total amount of the respective antimicrobial used to treat animals [2]. A high frequency of oxytetracycline resistance and multidrug resistance may be a result of the large amount of oxytetracycline and multiple other antimicrobials being used as feed additives to promote chicken growth as well as the excessive

use of these drugs in the veterinary setting.

A comparison of the results from the three countries revealed that *E. coli* isolates and enterococci from Vietnam exhibited a significantly higher prevalence for resistance to the majority of the antimicrobials tested. In addition, multidrug resistance was more frequently observed in isolates from Vietnam as compared with those from Indonesia and Thailand. In Vietnam, several studies have suggested that the high percentage of antimicrobial resistance in retail meats is caused by the unregulated and/or inappropriate use of antimicrobials in animal farming [11, 22, 25]. Our results suggest that the usage of antimicrobials in chickens in Southeast Asian countries, especially in Vietnam, is higher than that in developed countries [11, 22, 25].

In this study, the prevalence of bacteria resistant to fluoroquinolones was extremely high compared to that in developed countries [5, 16, 18]. The WHO considers both fluoroquinolones and third-generation cephalosporins to be critically important medicines for both humans and animals [7]. However, in developing countries, enrofloxacin (a fluoroquinolone) is often used not only as a medicine, but also as a feed additive to promote animal growth (personal communication with local veterinary officer). In the United States, the use of fluoroquinolone in poultry has been banned since 2004 [14]. In Japan, fluoroquinolones have been recommended for therapeutic use as second-line drugs in the veterinary field, since enrofloxacin was approved as a first-line veterinary fluoroquinolone in 1991. With increased globalization, the increased prevalence of fluoroquinolone-resistant bacteria in Southeast Asian countries places many other countries at high risk. Our current data suggest that the governments of Southeast Asian countries should strictly regulate the use of antimicrobials, particularly fluoroquinolones, in the treatment of food-producing animals.

In this study, three *E. faecalis* isolates and one *E. faecium* isolate exhibited low susceptibility to vancomycin (8 mg/*l*). Vancomycin-resistant enterococci (VREs) have previously been isolated from chicken meat that was imported into Japan from Thailand [15, 23]. While we did attempt to identify the genes responsible for vancomycin-resistance, such as *vanA* or *vanB*, we were unable to detect them. In Japan, VREs were not detected in food-producing animals in 1999 due to the ban on avoparcin (a vancomycin analogue) as a treatment for food-producing animals, which began in 1997 [19]. However, in Denmark during 2006, VREs were detected in 3% of *E. faecalis* samples obtained from pigs despite a ban restricting avoparcin use as a treatment for food-producing animals that began in 1995 [1]. Notably, avoparcin was still given to chickens in the three countries during this period (personal communication with local veterinary officer). Therefore, although VREs were not detected in this study, we advocate that continual surveillance for VREs in food-producing animals is important to accurately evaluate the future risk of infection for both humans and food-producing animals.

In this study, we determined the antimicrobial resistance of indicator bacteria obtained from chickens in three Southeast Asian countries. Our results show that monitoring the development and prevalence of antimicrobial resistance in developing countries is required. Ongoing surveys of antimicrobial resistance in developing countries are important, as the information obtained will help establish guidelines for the prudent use of antimicrobials.

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