Prevalence, antibiotic resistance and plasmid profiling of Salmonella in catfish (Clarias gariepinus) and tilapia (Tilapia mossambica) obtained from wet markets and ponds in Malaysia

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Prevalence, antibiotic resistance and plasmid profiling of *Salmonella* in catfish (*Clarias gariepinus*) and tilapia (*Tilapia mossambica*) obtained from wet markets and ponds in Malaysia

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ABSTRACT

The objectives of the study were to determine the prevalence, antibiotic resistance and occurrence of plasmids in *Salmonella* isolated from catfish (*Clarias gariepinus*) and tilapia (*Tilapia mossambica*) obtained from wet markets and ponds in Malaysia. A total of 172 samples (32 catfish carcass rinse, 32 catfish intestines, 32 tilapia carcass rinse, 32 tilapia intestines, and 44 water samples) were obtained from nine wet markets and eight ponds that were fed chicken offals, spoiled eggs, and commercial fish feed from 2008 to 2009. Seven *Salmonella* serovars were isolated from 9/32 catfish (28.1%), 14/32 tilapia (43.8%), and 11/44 (25%) water samples. These include *S.* Albany, *S.* Agona, *S.* Corvallis, *S.* Stanley, *S.* Typhimurium, *S.* Mikawashima and *S.* Bovis-mobificans. *Salmonella* isolates were resistant to chloramphenicol (C, 37.2%), clindamycin (Da, 100%), rifampicin (Rd, 90.7%), spectinomycin (Sh, 27.9%), and tetracycline (Te, 67.4%). The multiple antibiotic resistance index of *Salmonella* isolates ranged from 0.32 to 0.45 for catfish; 0.14 to 0.36 for tilapia; and 0.27 to 0.36 for water. The predominant antimicrobial resistance profiles of *Salmonella* serovars from catfish, tilapia and water were CDaRdTe (4/13), DaRdSh (4/19), and DaRdTe (6/11), respectively. The plasmids of *Salmonella* serovars isolated from catfish ranged from 23 to 80 kb; those for tilapia ranged from 6 to 90 kb; that for water ranged from 6 to 70 kb, respectively. The presence of plasmids represents a potential health hazard since plasmids can mediate the transfer of antibiotic resistance genes to other bacteria present in the fish, and aquaculture environment, which can also enter the food chain.

1. Introduction

Freshwater fish culture in Malaysia contributes 155,398.6 t valued at RM 760.3 million, representing 26.7% of the total production and constituted 27.2% of the overall aquaculture subsector (Department of Fisheries Malaysia, 2010). In Malaysia, freshwater fish is cultured using pond culture, ex-mining pool, freshwater cage, cement tank, canvas tank, and freshwater pen culture systems. The highest total freshwater fish production (59.7%) has been reared in the pond culture system and the types of fish cultured in this system are freshwater catfish (64.9%) and tilapia (18.2%) (Department of Fisheries Malaysia, 2010).

In the Asia–Pacific region, cultured fishes are fed by both commercial and homemade feeds (fresh feed material or farm feed material). According to FAO, homemade feeds are used to reduce cost of production (FAO, 2010a; New and Csavas, 1995). Homemade feed is usually made from chicken viscera, kitchen refuse, chicken bone, and other food waste materials (New and Csavas, 1995). Such feeds can be a source of pathogenic bacteria such as *Salmonella* spp. (Burr and Helmboldt, 1962; Lunestad et al., 2007) which can be transmitted to catfish and tilapia and ultimately to consumers.

Salmonella spp. are Gram-negative, rod-shape bacteria that cause salmonellosis. In humans, these pathogenic bacteria caused enteric fever (only if it is Typhi or Paratyphi) and acute gastroenteritis (Hohmann, 2001). The symptoms include mild to severe gastroenteritis, with an incubation period of 6–72 h (Hohmann, 2001). Outbreaks of salmonellosis due to fish consumption have been reported in several countries. For example, salmonellosis caused by smoked eel consumption, which was linked to fish farms in Italy has been reported in Germany (Fell et al., 2000). The U.S. Food and Drug Administration (US-FDA) has also linked the presence of *Salmonella* spp. in a variety of fishes and shellfishes (Brands et al., 2005; Duran and Marshall, 2005; Heinitz et al., 2000). Various hazards associated with cultured fish naturally originated from the environment or from human or animal activities. Fishes can serve as a vehicle of *Salmonella* transmission, which can be pathogenic to humans and have a high potential to transmit its antibiotic

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resistance gene to other pathogens via plasmids (Hradecka et al., 2008). The potential for antibacterials to cause development of resistance in fish pathogens is of concern worldwide (Schnick, 2001).

In Malaysia, data regarding the presence of different serovars of *Salmonella* in catfish and tilapia cultured in ponds with different types of feed are limited. Thus, present study was conducted to determine the prevalence, antibiotic resistance, and occurrence of plasmids in *Salmonella* serovars isolated from catfish (*Clarias gariepinus*) and tilapia (*Tilapia mossambica*) obtained from wet market and ponds (fed with chicken offals, spoiled eggs or commercial fish feed) in Malaysia.

2. Materials and methods

2.1. Samples

Catfish (*Clarias gariepinus*) samples were obtained from five local wet-markets and four ponds, while tilapia (*Tilapia mossambica*) samples were purchased from two wet markets, two hypermarkets and four ponds in Penang and surrounding Penang (Malaysia) (November 2008 to September 2009). Catfish and tilapia obtained from wet market originated from ponds in Malaysia. Wet markets were sampled on 4 and 5 different occasions for catfish and tilapia, respectively. Ponds were sampled 3 times for both catfish and tilapia. During each visit, 5–6 live catfish and tilapia were placed in sterile polypropylene bags and transported in polystyrene box to laboratory. In case of live tilapia, the polypropylene bag containing water and fish were flushed with oxygen and bag was tied using rubber band. The length of catfish and tilapia ranged from 50 to 60 and 20 to 25 cm, whereas the weight ranged from 750 to 1000 and 200 to 350 g respectively. Catfish purchased from wet markets were live and without skin lesions or of sunken eyes, while tilapia obtained from wet-markets were dead and the skin showed no lesions and the eyes were moist, bright, not sunken and the texture was firm. The fish was placed in sterile polypropylene bag, placed in polystyrene box containing crushed ice and the temperatures was between 4 and 8 °C during transportation. The samples were delivered and analyzed in the laboratory within 3 h. The fish was pooled and analyzed for the presence of *Salmonella* spp. Water samples were obtained from tanks in which live catfish were maintained in wet markets. Water from ponds where both catfish and tilapia were cultured, were also sampled. The ponds were selected based on the type of feed. The feeds used were chicken offals for catfish, spoiled eggs for tilapia and commercial fish feed.

2.2. Sample preparation

Isolation of *Salmonella* spp. was carried out by using 3 different preparation methods: Method A, B and C. In method A, one catfish was place in sterile plastic bag and rinsed with 200 mL of 0.1% (w/v) Peptone Water (PW, Oxoid, Baringstoke, Hampshire, UK). PW was poured onto the fish by using a sterile pipette. The fish was then gently massaged externally to ensure that the peptone water was uniform distributed on the surface of the fish. After through mixing, the rinse was then use to rinse another fish placed in another sterile bag, and this procedure was repeated 4–5 times. After rinsing 5–6 fish, the rinse was transferred into four sterile 50 ml polypropylene bottles and centrifuged for 15 min at 10,000 ×*g* (Kubota Model 6400, Tokyo, Japan) to obtain a pellet. In method B, the intestines (the contents of the intestines were not removed) of the rinsed catfish and tilapia were removed using a sterile knife and were pooled by using sterile forceps. The intestines with the contents were placed on a sterile tray wrapped in aluminium foil and chopped thoroughly with sterile knife. 25 g intestines were placed in a stomacher bag containing 225 mL 0.1% PW and homogenized using a stomacher (Interscience, France) for 2 min. The homogenate was divided equally, placed in 50 mL centrifuge tubes and centrifuged for 15 min at 10,000 ×*g* to obtain a pellet. The pellet was pre-enriched by re-suspending it in 10 mL Buffered Peptone Water (BPW, Merck KGaA, Darmstadt, Germany) and incubated at 37 °C for 24 h. In method C, 25 g of chopped intestines were placed in a stomacher bag and pre-enriched by homogenizing with 225 mL BPW using a stomacher for 2 min and incubated at 37 °C for 24 h.

2.3. Isolation and identification of Salmonella

After pre-enrichment, 1 mL portions were transferred into 10 mL of Rappaport and Vassiliadis broth (RV, Merck KGaA, Darmstadt, Germany) and incubated at 42 °C for 24 h. Following enrichment, 10 μL of the culture was streak-plated onto Rambach (Merck KGaA, Darmstadt, Germany), Xylose-Lysine-Tergitol 4 (XLT4, Merck KGaA, Darmstadt, Germany), Xylose Lysine Deoxycholate (XLD, Merck KGaA, Darmstadt, Germany), and Bismuth Sulfite Agar (BSA, Merck KGaA, Darmstadt, Germany) and was incubated at 37 °C for 24–48 h.

Twenty-five milliliters of water samples was pelleted by centrifuging at 10,000 ×*g* for 15 min, the pellet was re-suspended and pre-enriched in 10 mL of BPW and incubated at 37 °C for 24 h. Twenty-five milliliters of water samples was also directly pre-enriched by homogenizing in 225 mL of BPW, using a stomacher for 2 min and incubated at 37 °C for 24 h. After pre-enrichment, 1 mL of both pelleted and non-pelleted methods was enriched in 10 mL of RV broth and incubated at 42 °C for 24 h. After enrichment, 10 μL of culture was streak-plated onto Rambach, XLT4, XLD and BS agar plates and incubated at 37 °C for 24–48 h. Well isolated colonies giving typical reactions according to manufacturer's instructions were considered as presumptive *Salmonella* were purified by streaking onto nutrient agar plates (Merck KGaA (Darmstadt, Germany). Well isolated colonies were Gram stained and subjected to following biochemical tests: catalase, cytochrome oxidase, triple sugar iron, lysine iron, urease, indole and motility test. The biochemical test materials were obtained from Merck KGaA (Darmstadt, Germany). *Salmonella* was confirmed by using polyvalent O and H antisera (BD, Franklin Lakes, USA) according to the Bacteriological Analytical Manual (US-FDA, 2007). *Salmonella* isolates were serotyped by Institute for Medical Research, a WHO Reference Laboratory for *Salmonella*, in Kuala Lumpur. Molecular techniques such as 16S rRNA was not used in this study because the conventional microbiological methods are well established and are also used as reference methods.

2.4. Antibiotic susceptibility tests

The antibiotic susceptibility test was performed by using disc diffusion method. The isolates were tested against the antibiotics which included: azithromycin (Azm, 15 μg), ceftazidime (Cef, 10 μg), ciprofloxacin (Cip, 10 μg), clindamycin (Da, 2 μg), ceftriaxone (Cro, 5 μg), chloramphenicol (C, 10 μg), rifampicin (Rd, 30 μg), spectinomycin (Sh, 10 μg), sulphamethoxazole-trimethoprim 19:1 (Sxt, 25 μg), tetracycline (Te, 30 μg), tobramycin (Tob, 10 μg). The antibiotics discs were obtained from Oxoid (Baringstoke, Hampshire, United Kingdom). Cultures were grown overnight in Triptic Soy Broth (TSB, Merck KGaA, Darmstadt, Germany) and incubated at 37 °C. The overnight cultures were diluted to a turbidity of 0.5 on McFarland scale. The cultures were streaked on Mueller Hinton Agar (Oxoid, Baringstoke, Hampshire, United Kingdom) plates using a cotton swab. After 30 min, 3–4 antibiotic discs were placed on the plates and were incubated at 37 °C for 18–24 h. After the incubation period, the diameter of inhibition zones was measured and compared with interpretive chart proposed by the 'Performance Standards for Antimicrobial Disk Susceptibility Tests' and which were classified as resistant (CLSI, 2010).

The Multiple Antibiotic Resistances (MAR) index was determined for each pond according to the method described by Krumperman (1983). According to Krumperman (1983), MAR is defined as $a/(b \times c)$ where "*a*" represents the aggregate antibiotic resistance score of all the isolates, "*b*" is the number of antibiotics and "*c*" is the number of isolates from the fish. A MAR index value of less than or equal to 0.2 is considered to indicate the samples wherein antibiotics were seldom

or never used. MAR index value higher than 0.2 is considered to have originated from high-risk sources of contamination where antibiotics are very often used.

2.5. Detection of plasmids

Single colony of pure *Salmonella* culture was inoculated into 5 mL Luria-Bertani (LB, Merck KGaA, Darmstadt, Germany) and incubated in orbital shaker (with vigorous shaking) at 37 °C for 16–18 h. The overnight culture (1.5 mL) was centrifuged for 5 min at 1000 ×*g* to obtain pellets. Pellets were dried and subjected to plasmid DNA extraction and purification using Promega Wizard® plus Minipreps DNA Purification System (Promega, Madison, USA) by following the manufacturer's instructions (Anonymous, 2009). Plasmids were later loaded on 0.7% agarose gel and separated using horizontal gel electrophoresis system (GES Elite 300, Wealtec, Taipei, Taiwan). Plasmid DNA bands were visualized using UV transilluminator (UVItec Gel Imaging System, Cohasset MA, USA). The approximate molecular mass of each plasmid was determined by comparing with DNA Lambdha/Hind III Marker (Promega, Madison, USA).

2.6. Statistical analysis

The differences in the prevalence of *Salmonella* spp. between wet markets and between ponds were determined by using one-way ANOVA (SPSS version 13.0) at a significance level of $P<0.05$.

3. Results

3.1. Prevalence of Salmonella species isolated from catfish and tilapia

The prevalence of *Salmonella* spp. in catfish, tilapia and water samples obtained from wet markets and ponds is presented in Table 1. *Salmonella* was detected in 1/4 and 2/4 catfish samples obtained from wet markets A and E, respectively. *Salmonella* was not detected in catfish samples obtained from wet markets B, C, and D. *Salmonella* was isolated from 3/6 and 3/6 of catfish obtained from ponds fed with chicken offals and commercial feed, respectively. Statistical analysis showed no significant difference ($P>0.05$) between the catfish and water samples obtained from all sources.

Table 1
Prevalence of *Salmonella* spp. isolated from catfish, tilapia and water obtained from different wet markets and ponds in Malaysia.

Location	Number of positives samples		
	Catfish (%)	Tilapia (%)	Water (%)
Wet market			
A (Bukit Mertajam)	1/4 (25)	NA	1/4 (25)
B (Bagan Ajam)	0/4 (0)	NA	0/4 (0)
C (Nibong Tebal)	0/4 (0)	NA	0/4 (0)
D (Gelugor)	0/4 (0)	NA	1/4 (25)
E (Bayan Baru)	2/4 (50)	2/5 (40)	1/4 (25)[b]
F (Hypermarket S1)	NA[a]	1/5 (20)	NA
G (Hypermarket S2)	NA	0/5 (0)	NA
H (Chowrasta)	NA	0/5 (0)	NA
Ponds			
A1 (Chicken offal feed)	2/3 (66.7)	NA	3/3 (100)
A2 (Chicken offal feed)	1/3 (33.3)	NA	1/3 (33.3)
B1 (Commercial pellet feed)	2/3 (66.7)	NA	1/3 (33.3)
B2 (Commercial pellet feed)	1/3 (33.3)	NA	0/3 (0)
C1 (Spoiled egg feed)	NA	3/3 (100)	2/3 (66.7)
C2 (Spoiled egg feed)	NA	3/3 (100)	0/3 (0.3)
D1 (Commercial pellet feed)	NA	2/3 (66.7)	1/3 (33.3)
D2 (Commercial pellet feed)	NA	3/3 (100)	0/3 (0)
Total	9/32 (28.1)	14/32 (43.8)	11/44 (25)

a = not available; b = water obtained from catfish tank.

Salmonella was isolated from 2/5 and 1/5 tilapia samples obtained from a wet-markets E and F (Table 1). *Salmonella* was isolated from the intestines of tilapia (6/6) fed with spoiled eggs obtained from ponds C1 and C2. However, there was only 1/6 intestines of tilapia samples obtained from pond fed with commercial pellet (D2) which was positive for *Salmonella*. There was significant difference ($P<0.05$) in the prevalence of *Salmonella* in tilapia fed with spoiled eggs (ponds C1 and C2) and those fed with commercial feed (ponds D1 and D2). No significant difference ($P>0.05$) was found between the tilapia carcass rinse samples and water samples obtained from ponds C1, C2, D1 and D2.

Salmonella spp. were isolated from water samples obtained from three different wet markets and three ponds where catfish was retailed or reared. *Salmonella* serovars isolated from catfish and tilapia ponds are presented in Table 3. The distribution of different *Salmonella* serovars isolated from catfish and tilapia is shown in Table 3. The predominant *Salmonella* serovars were *S.* Albany (12/21) from catfish and *S.* Corvallis (15/22) from tilapia, respectively.

3.2. Antibiotic resistance among Salmonella isolated from catfish, tilapia and water

The antibiotic resistance among *Salmonella* serovars isolated from catfish, tilapia, and water samples obtained from wet markets and ponds is presented in Tables 2 and 3. All isolates were susceptible to azithromycin, ceftazidime, ciprofloxacin, ceftriaxone, sulphamethoxazole-trimethoprim, tobramycin and resistant to clindamycin. Most *Salmonella* serovars isolated from catfish and tilapia were resistant to chloramphenicol, rifampicin and tetracycline (Table 2).

The most common antibiograms of *Salmonella* isolated from catfish, tilapia, and water were CDaRdTe ($n=4$), DaRdSh ($n=4$) and DaRdTe ($n=6$), respectively (Table 3). The MAR index of *Salmonella* isolates from ponds that were fed with chicken offals and spoiled eggs was relatively higher compared to those isolated from ponds fed with commercial feed (Tables 2 and 3). MAR index of *S.* Albany, *S.* Agona, *S.* Corvallis, *S.* Stanley and *S.* Typhimurium isolated from catfish ranged from 0.18 to 0.46. These serovars were resistant against 2–5 different antibiotics (Table 3). In contrast, the MAR index of *S.* Corvallis, *S.* Mikawashima, *S.* Bovis-mobificans, *S.* Agona and *S.* Typhimurium isolated from tilapia ranged from 0.18 to 0.36, and were resistant against 2–4 different types of antibiotics (Table 3).

3.3. Presence of plasmids

The plasmid sizes of *Salmonella* serovars isolated from catfish, tilapia and water ranged from 23 to 80 kb, 6 to 90 kb and 6 to 80 kb, respectively (Table 3). Five out of nine isolates isolated from ponds fed with chicken offals and water samples harbored plasmids. Plasmids were not detected in isolates from catfish fed with commercial feed (Table 3).

The isolates from ponds that used spoiled eggs harbored plasmid sizes ranging from 5 to 74 kb for tilapia and 23 to 40 kb for water samples. These plasmids were observed in 11/12 tilapia and water samples. The isolates isolated from tilapia and water fed with commercial feed harbored plasmids with sizes ranging from 6 to 43 kb (5/7) (Table 3).

4. Discussion

4.1. Prevalence of Salmonella in catfish, tilapia and water

Salmonella serovars were isolated from catfish, tilapia and water. Ellermeier and Slauch (2006) revealed that cold-blooded animals such as catfish and tilapia (FAO, 2010a) are potential hosts for *Salmonella* species (Baker and Smitherman, 1983; Pal and Marshall, 2009). The prevalence of *Salmonella* spp. in catfish and tilapia fed with chicken

Table 2
Number (%) of *Salmonella* isolates isolated from catfish, tilapia and water obtained from wet markets and ponds resistant to chloramphenicol (C), clindamycin (Da), rifampicin (Rd), spectinomycin (Sh) and tetracycline (Te).

Antibacterial agents	Number (%) resistant isolates										
		Catfish					Water				
	Total isolates	Wet market	Pond[a] A1	Pond[a] A2	Pond[b] B1	Pond[b] B2	Wet market	Pond[a] A1	Pond[a] A2	Pond[b] B1	Pond[b] B2
	(*n* = 21)	(*n* = 4)	(*n* = 4)	(*n* = 1)	(*n* = 3)	(*n* = 1)	(*n* = 3)	(*n* = 3)	(*n* = 1)	(*n* = 1)	(*n* = 0)
Chloramphenicol (C)	11 (52.4)	2 (50)	1 (25)	1 (100)	3 (100)	1 (100)	1 (33.3)	1 (33.3)	1 (100)	0 (0)	NA
Clindamycin (Da)	21 (100)	4 (100)	4 (100)	1 (100)	3 (100)	1 (100)	3 (100)	3 (100)	1 (100)	1 (100)	NA
Rifampicin (Rd)	20 (95.2)	4 (100)	4 (100)	1 (100)	2 (66.7)	1 (100)	3 (100)	3 (100)	1 (100)	1 (100)	NA
Spectinomycin (Sh)	6 (28.6)	1 (25)	3 (75)	0 (0)	0 (0)	0 (0)	1 (33.3)	1 (33.3)	0 (0)	0(0)	NA
Tetracycline (Te)	17 (81)	2 (50)	3 (75)	1 (100)	3 (100)	1 (100)	3 (100)	2 (66.6)	1 (100)	1 (100)	NA
MAR Index		0.32	0.32	0.45	0.33	0.36	0.33	0.30	0.36	0.27	NA
		Tilapia					Water				
	Total isolates	Wet market	Pond[c] C1	Pond[c] C2	Pond[b] D1	Pond[b] D2	Pond[c] C1	Pond[c] C2	Pond[b] D1	Pond[b] D2	
	(*n* = 22)	(*n* = 3)	(*n* = 5)	(*n* = 5)	(*n* = 2)	(*n* = 4)	(*n* = 2)	(*n* = 0)	(*n* = 1)	(*n* = 0)	
Chloramphenicol (C)	5 (22.7)	2 (66.6)	1 (20)	0 (0)	1 (50)	1 (25)	0 (0)	NA[d]	0 (0)	NA	
Clindamycin (Da)	22 (100)	3 (100)	5 (100)	5 (100)	2 (100)	4 (100)	2 (100)	NA	1 (100)	NA	
Rifampicin (Rd)	19 (86.4)	3 (100)	5 (100)	4 (80)	2 (100)	2 (50)	2 (100)	NA	1 (100)	NA	
Spectinomycin (Sh)	6 (27.3)	2 (66.6)	0 (0)	2 (40)	2 (100)	0 (0)	0 (0)	NA	0 (0)	NA	
Tetracycline (Te)	12 (54.5)	2 (66.6)	3 (60)	1 (20)	2 (100)	1 (25)	2 (100)	NA	1 (100)	NA	
MAR Index		0.36	0.27	0.22	0.32	0.14	0.27	NA	0.27	NA	

a = Pond with chicken offal feed system; b = Pond with commercial fish feed system; c = Pond with spoiled eggs feed system; d = not available. Azythromycin (Azm), ceftazidime (Cef), ciprofloxacin (Cip), cefriaxone (Cro), sulphamethoxazole-trimethoprim (Sxt) and tobramycin (Tob) were susceptible.

offals or spoiled eggs was relatively higher than those fed with commercial fish feed. Similar results were observed in water from ponds (Table 1). Feed serves as a niche for *Salmonella* spp. growth in fish and water (Lunestad et al., 2007). Feed made from chicken offals, spoiled eggs and commercial fish feed could transfer *Salmonella* spp. to the aquaculture environment. The spoiled eggs used as feed in this study were spoiled unfertilized or fertilized chicken eggs with a nearly developed embryo inside. Prevalence of *Salmonella* spp. in chickens, eggs and feed has been reported by many researchers (Otomo et al., 2007; Singh et al., 2010; Tooru et al., 2001; Veldman et al., 1995).

Water used in the ponds was from different sources. Most of the catfish and tilapia ponds used either stream or ground water, which might transfer *Salmonella* spp. to the fish. Previous studies reported that stream water (Huang et al., 2011) and ground water (Li et al., 2009) were contaminated with *Salmonella* spp. Unlike catfish ponds, tilapia ponds are 18–50 m deep, which made cleaning and changing of water more difficult before rearing. This condition increased the presence of *Salmonella* spp. in water. Amagliani et al. (2012) revealed that *Salmonella* can enter the aquatic environment through poor sanitation and inappropriate disposal of human and animal wastes.

Table 3
Antibiogram and plasmid profile of *Salmonella* serovars isolated from catfish, tilapia and water obtained from wet markets and ponds.

Salmonella serovars	Source	No. of isolates resistant to antibiotics	Antibiogram	No. of isolates harboring plasmids	Plasmid size (kb)	MAR index
Catfish						
S. Albany	Pond A1, A2	3	CDaRdShTe	2	20; 23	0.46
	Pond A2,B1,B2, A	5	CDaRdTe	2	23	0.36
	E	1	DaRdShTe	1	70	0.36
	Pond B1	1	CDaTe	0		0.27
	Pond B1	1	DaRdTe	2	23	0.27
	E	1	DaRd	0		0.18
S. Agona	Pond A1	3	DaRdTe	2	23	0.27
S. Corvallis	A	1	CDaRdTe	1	15, 23, 40	0.36
	A, D	2	DaRdTe	2	23, 80	0.27
S. Stanley	Pond A1	1	DaRdSh	0		0.27
	Pond A1	1	DaRd	0		0.18
S. Typhimurium	E	1	CDaRdShTe	1	23,70	0.46
Tilapia						
S. Corvallis	F	1	CDaRdTe	0		0.36
	E	1	DaRdShTe	1	23, 40	0.36
	Pond C2	1	CDaRd	1	23, 40	0.27
	Pond C2,C1	4	DaRdSh	4	6, 23, 40	0.27
	Pond C1,D1	6	DaRdTe	6	5, 30, 40 74	0.27
	Pond C2	2	Da	2	23,43	
S. Mikawashima	Pond D2	1	CDaRdTe	1	23	0.36
	Pond D2	1	DaRd	0		0.18
	Pond D2	1	Da	0		
S. Bovis-mobificans	Pond D1	1	CDaRdTe	1	23, 30	0.36
	Pond D1	1	DaRdTe	1	6, 23, 40	0.27
S. Agona	E	1	CDaRdSh	1	50	0.36
S. Typhimurium	Pond C2	1	DaRdTe	0		0.27

C = chloramphenicol; Da = Clindamycin; Rd = rifampicin; Sh = spectinomycin; Te = tetracycline.

Winfield and Groisman (2003) stated that *Salmonella* can reach soil and aquatic environments, survive for over long periods and passage into new host.

Water pollution is caused by several factors. Studies have shown that water pollution can be due to organic material, overstocking of fish or other factors (Amagliani et al., 2012; Wyatt et al., 1979). According to Iwamoto et al. (2010), polluted water can contribution to the colonization of fish by *Salmonella* spp. and hence become a potential source Salmonella transmission to humans (Iwamoto et al., 2010). The poor hygienic standards of run-off waters from human sewage, livestock farming or industry can also promote *Salmonella* in aquaculture system (Martinez-Urtaza and Liebana, 2005).

According to FAO (2010b) *S.* Albany, *S.* Agona, *S.* Corvallis, *S.* Stanley, *S.* Bovis-mobificans, and *S.* Typhimurium were present in fish, fisheries product, and aquaculture environments. In this study, *S.* Mikawashima was isolated from fish and aquaculture system and the presence of this serovar has not being reported by other researchers. The presence of *S.* Albany, *S.* Agona and *S.* Stanley in catfish fed with offals and *S.* Corvallis in tilapia fed with spoiled eggs suggests that these serovars might have originated from chicken offals and eggs. The presences of these serovars in poultry and eggs has been reported by other researchers (Modarressi and Thong, 2010; Otomo et al., 2007; Tooru et al., 2001).

The presence of *S.* Typhimurium in catfish and tilapia (Table 3) is of concern because *S.* Typhimurium can adversely affect human health. According to Stan Bailey and Maurer (2001), 70% of all the reported cases of salmonellosis world wide are due to *S.* Typhimurium and *S.* Enteritidis.

4.2. Antibiotic resistance among Salmonella serovars isolated from catfish, tilapia and water

Antibiotics are used for treatment and as growth promoters in the animal husbandry and aquaculture which lead to development of resistance (Serrano, 2005) among environmental species. In our study, we observed that all isolates were resistant to clindamycin (100%) and susceptible to azithromycin, ceftazidine, ciprofloxacin, ceftriaxone, sulphamethoxazole-trimethoprim. Resistance to clindamycin, especially in these farming systems, likely reflects the excessive use of this drug for the treatment in catfish farming. In Malaysia, the use of antibiotics is not regulated effectively and enforcement is weak or rather non-existent.

In this study, antibiotic resistance to chloramphenicol and tetracycline in *Salmonella* isolates was observed. Acquired resistance to tetracycline and chloramphenicol has been attributed to the extensive use of antibiotics in aquaculture farming in some Asian countries (Mohamed et al., 2000) and poultry (Gyles, 2008). Sapkota et al. (2008) reported that of the top 13 aquaculture-producing countries (excluding Egypt and North Korea), 92% used oxytetracycline and 69% used chloramphenicol.

Doublet et al. (2004) reviewing the work of other researchers, described the *Salmonella* Genomic island 1(SGI1) which contains an antibiotic resistance gene cluster conferring the common multi-drug resistance profile ApCFfSmShSuTe (i.e., ampicillin [Ap], chloramphenicol [C], florfenicol [Ff], streptomycin [Sm], spectinomycin [Sh], sulfonamides [Su], and tetracycline [Te]) of epidemic multidrug-resistant serovar Typhimurium DT104. SGI1 has been identified in *S. enterica* serovars Agona, Paratyphi B, and Albany, indicating the horizontal transfer potential of SGI1. SGI1-carrying serovar Agona, Paratyphi B, and Albany strains were isolated from different animal species, such as poultry in Belgium, tropical fish from Singapore, and food fish imported from Thailand in France, respectively (Doublet et al., 2004). Recently, they reported the first human case infected by a serovar Agona strain harboring SGI1-A.

Almost all *Salmonella* isolates isolated from catfish (20/21) and tilapia (19/22) were resistant to rifampicin (Table 2). McPhearson et al. (1991) reported that the resistance to antibiotics in Gram-negative bacteria from cultured catfish and aquaculture ponds was higher in ponds undergoing antimicrobial therapy or with a history of recent treatment than in ponds without recent antimicrobial treatment. In a previous study, Radu et al. (2000) reported that all strains of *S.* Enteritidis isolated from tilapia retailed at wet-markets in Selangor, Malaysia were susceptible to rifampicin.

The MAR index ranged from 0.18 to 0.46 (Table 3) for all the *Salmonella* serovars. The emergence of *Salmonella* serovars with high MAR index suggests that these serovars have originated from environments where antimicrobials are often used as therapeutic or as growth promoters in animal feeds (Krumperman, 1983; Singh et al., 2010). Multiple drug resistant *Salmonella* isolates have been suggested to be more virulent than nonmultiple drug resistant *Salmonella* isolates (Fluit, 2005; Foley and Lynne, 2008). *Salmonella* resistant to one or more antibiotics have been reported by many investigators (Foley and Lynne, 2008; Pan et al., 2010; Tsai and Hsiang, 2005).

A total 35/43 of *Salmonella* isolates harbored plasmids with sizes ranging from 5 to 74 kb (Table 3). Most of the isolates harbored more than one plasmid. Horizontal transfer of resistance genes on plasmids has been demonstrated between bacteria in the water of fish ponds (Aoki, 1997) and in marine sediments (Stewart and Sinigalliano, 1990). Plasmids in *Salmonella* spp. have been reported to transfer antibiotics resistance and virulence traits (Carattoli, 2003; Hradecka et al., 2008). The present study found the presence of plasmids on serovars Albany, Agona, Corvallis, Typhimurium, Mikawashima, Bovis-mobificans isolated from catfish, tilapia and water.

5. Conclusions

Feeds such as chicken offals and spoiled eggs can be potential source of *Salmonella* spp. and the high risks associated with the dissemination of antibiotic resistance genes among bacteria associated with catfish, tilapia and environment of aquaculture systems. This should be considered seriously by legal authorities to make appropriate laws and regulations.

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