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Prevalence of *Salmonella* spp. from Catfish (*Clarias gariepinus*) by using improvement isolation methods

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Abstract. Catfish is an important freshwater fish used in food supply may be infected by pathogenic bacteria such as *Salmonella* spp. Due to its public health implications; an enhanced method of isolation of *Salmonella* spp. is desirable. A total of 60 samples (15 of whole-body of catfish, 15 of gills, 15 of intestines and 15 of water samples) collected from wet market in Penang (Malaysia) were examined. The aim of this study was to compare a pellet method to the conventional method (non-pellet method) of isolation of *Salmonella* spp. from different parts of catfish (*Clarias Gariepinus*) and water samples. In this study, the pellet method was assessed and compared to conventional method (non-pellet method). Three selective agars, Xylose Lysine Deoxycholate Agar (XLD), Xylose-Lysine-Tergitol 4 (XLT4), Bismuth Sulfite Agar (BSA), were used. Pellet method found to show significant difference ($p<0.05$) on a total of 15 catfish samples. Higher prevalence obtained in pellet method was 53.33%, 80%, 40% growth on XLD, XLT4 and BSA, respectively. By using non-pellet method, the prevalence was 46.67%, 53.33%, 13.33% on XLD, XLT4 and BSA, respectively. *Salmonella* spp. presented on whole-body of catfish, gills, intestine and water for 80%, 40% 20% and 6.67%, respectively. This result indicates that the pellet method can isolate *Salmonella* sp. higher compared to non-pellet method.

Keywords: Salmonella spp., isolation, pellet method, catfish

1. Introduction

Salmonella spp*.,* a pathogenic, rod shaped, gram negative, had been frequently defined as opportunistic and potential pathogenic bacteria of water bodies in warm climate zones which pose a great risk on human health [9; 14]. This pathogenic bacteria has been isolated from freshwater fish such as catfish [3, 7, 17]. Catfish has emerged to be an important aquaculture production for food supply. The average contribution of aquaculture per capita fish available for human consumption rose from 14 percent in 1986, to 30 percent in 1996 and to 47 percent in 2006, and it can be expected to reach 50 percent in the next few years [6]. *Salmonella* spp. infections can be life-threatening, especially for the very young, the elderly, and for persons with impaired immune systems. It is clear that the contamination of *Salmonella* sp. with or without antimicrobial resistance became a food safety problem. Thus, it is important to develop some alternative methods to isolate this bacteria. The aim of this study is compare a pellet method to the conventional method of isolation of *Salmonella* spp. from different part of catfish (*Clarias Gariepinus*) and water.

2. Material and Method

2.1. Samples

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Catfish samples were each taken from 3 wet markets in 3 different regions in Penang (Malaysia). The samples were purchased from the same vendor in between November 2008 – January 2009. About 5-6 live fishes were pooled and brought in sterile bag with aeration during transportation. It was kept at 4-8^{o}C and was proceeded within 6 hours. Both the catfish and the water were examined.

2.2. Isolation of *Salmonella* spp.

Catfish was separated aseptically in different parts (gills, intestine, and whole-body part of catfish) and chopped with sterilized knife. Next 25 grams of each parts was blended (Stomacher 400) with 225 ml Buffered Peptone Water (BPW, Merck) for 2 min. Pellet were obtained by centrifuging (Kubota 6400) at 20^{o}C, 10,000 x g RPM, 15 minutes for fish sample and water sample (4^{o}C). The pellet was then dissolved into 10 ml of BPW and incubated at 37^{o}C for 24 hours. Afterwards, 1 ml of BPW was transferred onto Salmonella Enrichment Broth according to Rappaport and Vassiliadis (RV broth-Merck) and incubated at 42^{o}C for 24 hours.

The enrich inoculum was streaked onto Xylose Lysine Deoxycholate (XLD-Merck), Xylose-Lysine-Tergitol 4 (XLT4 Merck) and Bismuth Sulfite Agar (BSA-Merck) and were incubated at 37^{o}C for 24-48 hours. *Salmonella* spp. grew as pink with/without black centre on XLD, black colony on XLT4 and grey-black with metallic sheen coloured on BSA. Further gram stain and biochemical tests were carried out. These included catalase, cytochrome oxidase, microscopic observation, cell motility, TSI Triple Sugar Iron (Merck), Lysine Iron Agar (Merck), urease, phenol red dulcitol broth, indole test, Methyl Red-Voges Proskauer , Simmons citrate agar, motility test on SIM medium (Merck), serological polyvalent flagellar (H) test (BD) and serological somatic test (BD) are described in the Bacteriological Analytical manual [7]. *Salmonella* spp. and *Escherichia coli* from Food Microbiology Laboratory, University Sains Malaysia were used as control organisms.

2.3. Statistical analysis

A 3x2 factorial design was used to carry out to study the effect of isolation method (chopped-pellet and chopped-non pellet as a conventional method) and media (BSA, XLD, XLT4) on *Salmonella* spp. isolation. The experiment was run randomly and replicated 5 times. The samples were collected from 3 markets and the total run was 90. Six fish was used in each run. Another experiment with single factor was carried out to study the effect of market [10]. Tukey's test was carried out for multiple comparisons. Analysis of variance (ANOVA) was used to analyze the results obtained from this experiment using SPSS software for Windows version 12.

3. Results

In this study were obtained using the pellet methods in 53.33% (8/15), 80.00% (12/15), 40.00% (6/15) of the first sa,ples on XLD, XLT4 and BSA respectively. The pellet method gave isolates higher than non-pellet method in range 0 - 26.67% (Table 1).

Table 1. Number of confirmed *Salmonella* spp. in isolated from catfish

Media	BSA		XLD		XLT4	
Isolation Method	Pelet	Non Pelet	Pelet	Non Pelet	Pelet	Non Pelet
Whole-body (N=15)	6 (40.00%)	2 (13.33%)	8 (53.33%)	7 (46.67%)	12 (80.00%)	8 (53.33%)
Gills (N=15)	2 (13.33%)	1 (6.67%)	4 (26.67%)	3 (20.00%)	6 (40.00%)	2 (13.33%)
Intestine (N=15)	0 (0.00%)	0 (0.00%)	1 (6.67%)	2 (13.33%)	3 (20.00%)	2 (13.33%)
Water (N=15)	1 (6.67%)	0 (0.00%)	1 (6.67%)	0 (0.00%)	1 (6.67%)	1 (6.67%)

Note : BSA (Bismuth Sulfite Agar), XLD (Xylose Lysine Deoxycholate Agar), XLT4 (Xylose-Lysine-Tergitol 4).

Isolation methods and media exhibited a significant effect on *Salmonella* sp, while the interaction was insignificant. This indicates that the performance of each method and each media were not the same to others.

Based on Tukey's test we found that BSA was observed different compared to XLT4 for whole-body and intestine of catifish. This is because the sensitivity of each medium was not same. The result of this study found that sensitivity of XLT4 is higher than BSA.

The effect of different markets was analyzed by ANOVA. The results showed insignificant different between markets. This indicates that the distribution of *Salmonella* sp. in each market was the same.

Analysis of variance (ANOVA) showed insignificant on different market. It meant the samples from different markets were similar to other which has been contaminated by *Salmonella* sp. Tukey's test showed that all market showed in the same subset. This indicate that incidence of *Salmonella* sp. is almost similar on comparison between market.

Salmonella spp. was found from different parts of catfish, mostly in whole part of catfish (80%). Next, the Gills and intestines were contaminated for 40% and 20%, respectively. Water, potential agent of transmission of *Salmonella* spp., was contaminated to a level 6.67%.

Table 6. Number of confirmed *Salmonella* spp. positive catfish at each market

Sample	Market A (n=5)	Market B (n=5)	Market C (n=5)
Whole-body	3/5	5/5	4/5
Gills	2/5	2/5	2/5
Intestine	0/5	2/5	1/5
Water	1/5	1/5	1/5

BSA (Bismuth Sulfite Agar), XLD (Xylose Lysine Deoxycholate Agar), XLT4 (Xylose-Lysine-Tergitol 4).

This study found that market B gave the highest positive *Salmonella* spp. in whole-body of catfish. It will be important data to study further more on traceability of this incidence. Analysis of variance (ANOVA) showed insignificant difference among the market. It meant the samples from different markets were similar to other which has been contaminated by *Salmonella* sp. Water of catfish showed less to be contaminated by these pathogenic bacteria.

4. Discussion

The higher isolates obtained by using pellet method can be explained as centrifugation can sediment bacteria cell. Kirschner [10] revealed that bacterial cell can be separated by using centrifugation. Thus, the chance of isolating the bacteria is higher than non-pellet method.

Statistical analysis also showed that type of media was significantly different for *Salmonella* spp. isolation. Ruiz [16] revealed that the isolation of pathogenic bacteria from a sample requires the use of culture media which fulfill two attributes: an inhibiting effect on the growth of the largest possible amount of saprophytes and a discriminating capacity which allows it to be recognized among the other species which are also capable of growth on the medium. In this study, XLT4 seem to give more isolates than BSA. Dusch [4] , Voetsch *et al* [18] and Dworkin [5] reported that sensitivity of XLT4 and BSA were 86.6% and 70%, respectively. Michael [13] revealed that XLT4 presented better conditions for isolation of *Salmonella* sp. colonies, reducing the number of false-positives. Consequently, better selectivity and better indicator system of XLT4 resulted in greater detection of *Salmonella* spp., mainly when streaked from a selective enrichment that avoids overgrow of competitors.

Isolation method was also significantly different on *Salmonella* spp. isolation from catfish. That meant pellet method can be used as support method for non-pellet method as a standard method of US-FDA. In this study, the pellet methods was 0 - 26.7% more sensitive in isolating compared to the conventional method.

Sources of *Salmonella* spp. contamination were found in different part of catfish's body. Hatha [8] reported that these bacteria would exist on catfish's skin, gills and intestine and the most potential reservoir of *Salmonella* spp. was the intestine. Thus, it is strongly recommended to avoid cross-contamination of other tissued by digestive track during handling and preparation.

Results also indicated that catfish can be contaminated by the water used to keep them alive. This is in agreement with the studies of Alcaide [1] which revealed that *Salmonella* spp. is a potential pathogen for human and fish which can occur in the freshwater farm environment. This will be a route of transfer for the *Salmonella* spp. in environment from fish and finally to human. It means water used of catfish can be a potential agent to transfer these pathogenic bacteria from one to another and vice versa.

The highest *Salmonella* spp. incidence in whole-body of catfish was found from market B (100%). It can be interesting for the future study in order to know the route of *Salmonella* spp. transmission from pond to the next chain food supply. Thus, using the pellet method and proper media (XLT4) was used get more isolates of *Salmonella* sp. in its detection in catfish; ensure food safety from farm to fork.

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