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OCCURRENCE OF *SALMONELLA SPP* FROM FRESH FISH (Tilapia Nilotica Linn) USING IMPROVED ISOLATION METHODS

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ABSTRACT: *Fresh fish (Tilapia Nilotica Linn) is a very important source of protein to the population in our country; this is of importance when other sources of animal protein are in short-supply. This freshwater fish may harbor salmonella spp. Which may be a source of pathogen to human hence, this study is important due to the public health implications. A total of 90 samples (30 whole freshwater fish, 30 intestines and 30 gills) were collected from different open market in Aba, South, Aba North and Osisioma Local Government Area all in Abia State, and examined. The objective of this study was to compare between non-pellet method and pellet method of isolation of salmonella spp. from different parts of freshwater fish. In this study, the pellet method was evaluated and compared with the non-pellet. Three selective agars used for the purpose of this study namely: Salmonella-shigella Agar (SSA) xylose Lysine Deoxycholate Agar (XLD) and Bismuth sulfite Agar (BSA). There was significance difference (P<0.05) with the pellet method on the 30 Tilapia sample. The frequency of occurrence in pellet method was 66.66%, 50.00% and 26.66% growth on SSA, XLD and BSA while non-pellet method presented occurrence of 33.33%, 23.33% and 13.30% for SSA, XLD and BSA respectively. It was observed that salmonella sp was found more on the whole body of Tilapia nilotica than the gill and intestine presenting 66.66%, 50.00% and 20.00% respectively. The result confirms that pellet method isolated higher number of salmonella sp than non – pellet method.*

Key words: *Tilapia nilotica, salmonella sp., Isolation, "pellet and non-pellet method"*

ORIGINAL ARTICLE

INTRODUCTION

Salmonella spp. is a pathogenic, nod shaped, gram negative pathogenic bacteria of water bodies in warm climate zones which pose a great risk on human health (Heinitz et al., 2000). Salmonella cause a wide range of human diseases such as enteric fever, bacteramia and gastroenteritis. Gastroenteritis has the greatest adverse effect on children's growth and development (Black et al., 1984). The majority of 1.3billion annual cases of salmonella cause human gastroenterities resulting from ingestion of contaminated food products such as undercooked beef, pork, eggs shell fish and fish (Esaki et al., 2004).

Thampuren et al. (2005) reported that the microbial quality of the tilapia indicated that all tissues were contaminated with salmonella and fecal coli form. Salmonella can be disseminated as a result of water currents, underground springs and rain runoff carrying contaminated material (Abdel-Monem et al., 1990). Human infections by these fish pathogen are usually through contact with infected fish while handing them, water or other constituents of fish life environment (Acha et al., 2003). This pathogenic organism has been isolated from freshwater fish such as Tilapia nilotica Linn (D'Aoust et al., 1992). Tilapia is an important aquaculture production for food supply. Since on a global scale, fish and fish products are the most important source of protein and it is estimated that more than 30% of fish for human consumption comes from aquaculture (Hastein et al., 2006). *Salmonella spp.* Infections can be life threatening, especially for the very young, the elderly, and for persons with impaired.

Systems (Morales et al., 2004) showed that the presence of *Listeria sp*. and *Salmonella sp*. from the external surface of tilapias were remarkable. It is clear that the contamination of *Salmonella sp.* with or without antimicrobial resistance become a food safety problem. Hence, it is very necessary to develop some alternative methods to isolate the bacteria. The aim of this study is to compare a pellet method to a non-pellet method of isolation of *salmonella sp.* from different part of tilapia fish (Tilapia nilotica Linn).

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MATERIAL AND METHOD

Sample

Tilapia nilotica Linn samples were obtained from three markets located in three different local government areas. The samples were obtained from July 2010 to January 2012. An average of 5 live fishes was bought and transported to Michael Okpara University of Agriculture, Veterinary Laboratory in well ventilated sterile plastic container within 4 hours. The Tilapia fish was thereafter examined.

Sample processing

With gloved hands and sterilized knife, the Tilapia fish was severed into parts (intestine, gill and whole body of the Tilapia fish). 20 grams of each part was grinded (stomacher 400) with 225ml Buffered Peptone Water (BPW) for 3min. pellet were obtained by centrifugation at 20^0C, 10,000 x g RPM, for 15minutes for fish sample. The pellet was then dissolved into 10ml of BPW and incubated at 37^0C for 24hr 1ml of BPW was later transferred onto salmonella enrichment Broth according to Rappaport and Vassiliadis (RV broth-Merck) and incubated at 42^0C for 24hrs.

The inoculum was later streaked onto *salmonella shigella Agar* (SSA Difco), Xylose-Lysine Deoxycholate (XLD Difico) and Bismuth sulfite Agar (BSA – Difco) and were incubated at 37^0C for 48hrs. In SSA *Salmonella spp.* were seen as white or yellow with black spot centrally, in XLD, *Salmonella spp.* grew as pink color with black centre while in BSA salmonella colony grew as grey black with metallic sheen color. Biochemical tests and Gram stain was carried out. These include. Oxidase, catalyze, phenol red, ducitol, indole, Simon's citrate, methyl-red-Voges Proskauer, motility tests. Others are serology using polyvalent H and somatic antigens as described in Bacteriological Analytical Manuel (FDA, 2007). The control was *Salmonella spp.* and *Esherichia Coli* obtain from Veterinary Microbiology Laboratory of Michael Okpara University of Agriculture, Umudike. Nigeria.

Statistical analysis

The experimental design used was that of factorial 3x2 to evaluate the effect of isolation method (chopped – pellet and chopped – non – pellet method) on SSA, XLD and BSA media for salmonella isolation. Random sampling which was repeated 4 times. The total sample run was 120 obtained from 3 different markets. 5 fish was used in each run. Turkey's test was carried out for multiple comparisons (Kirschner et al., 1999). Why analysis of variance. (ANOVA) was used to analyze the result obtained.

RESULT

This study showed that pellet methods presented 66.66% ($^{20}/_{30}$), 50.00% ($^{15}/_{30}$), 26.00% ($^{8}/_{30}$) of the whole fish sample on SSA, XLD and BSA respectively. The pellet method obtained higher isolates than non-pellet method.

Table 1 - Number of conformed *salmonella spp.* Isolated from Tilapia

Media	SSA		XLD		BSA	
Isolation method	Pellet	Non pellet	Pellet	Non pellet	Pellet	Non pellet
Intestine (N = 30)	6	4	2	4	0	0
Gills (N = 30)	12	4	8	6	4	2
Whole fish (N = 30)	20	10	15	07	08	4

Table 2 - The Number of conformed *Salmonella spp.* isolated from Tilapia in percentage

Media	SSA		XLD		BSA	
Isolation method	Pellet	Non pellet	Pellet	Non pellet	Pellet	Non pellet
Intestine (N = 30)	20.00%	13.30%	6.66%	13.30%	0.00%	0.00%
Gills (N = 30)	40.00%	13.30	26.66%	20.00%	13.30%	6.66%
Whole fish (N = 30)	66.66%	33.33%	50.00%	23.33%	26.00%	13.30%

SSA= (*Salmonella Shigella Agar*). XLD= (Xylose Lysine Deoxycholate Agar). BSA= (Bismuth Sulfite Agar).

Table 3 - The number of *Salmonella spp.* positive in Tilapia at different market

Sample	Aba South (Market) (n – 4)	Aba North (Market) (n – 4)	Osisioma (Market) (n – 4)
Intestine	$^{2}/_{4}$	$^{1}/_{4}$	$^{0}/_{4}$
Gills	$^{2}/_{4}$	$^{2}/_{4}$	$^{1}/_{4}$
Whole-fish	$^{4}/_{4}$	$^{3}/_{4}$	$^{2}/_{4}$

SSA (Salmonella shegella Agar), XLD (xylose lysine deoxycholate Agar), BSA (Bismuth Sulfite Agar)

The method of isolation and media used contributed to the effect of *Salmonella sp* significance. This proves that different media produce different results and performance. The result obtained of the intestine and whole body fish differ because of the sensitivity of different media that was used. From this study, SSA media was more

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sensitive than BSA. There was no significance difference between markets in the three Local Government Area as analyzed by ANOVA. This shows that the distribution of *Salmonella sp.* in the three market more about the same. Turkey's result shows all market were evident in the same subset. This means that occurrence of *Salmonella sp.* is similar when compared between market. *Salmonella spp.* was present in all parts of the Tilapia fish especially the whole body, gills and intestine represented by (66. %), (50%) and (26%) respectively.

DISCUSSION

In this study, larger number of isolates were obtained using the pellet method than the non pellet method of isolation. This was supported by (Kirschiner et al., 1999). He showed that the chance of isolating the bacteria cell by centrifugation is higher in pellet method than non – pellet method. The type of media used was seen to be significantly different for *Salmonella sp.* and this was reflected in the statistical analysis. The isolation of pathogenic bacteria from a sample requires the use of culture media. This is in agreement (Ruiz et al., 2004). In the above study, SSA gave more bacterial isolates than the other two XLD and BSA (Dutch et al., 1995). He reported that the sensitivity of SSA and BSA were 76.6% and 50.0% respectively. (Michael et al., 2003) showed that SSA presented better conditions for isolation of salmonella sp. colonies, hence eliminating the volume of false positives. As a result, the better selectivity of the media is responsible for the greater detection of *Salmonella sp:* majorly when streaked from a selective enrichment that eliminates overgrowth of competitors the method of isolation was largely responsible for the significant difference on *Salmonella sp.* isolated farm tilapia fish. This implies that the pellet method could equally serve as support method for non – pellet method as a standard method of US -FDA.

The pellet – methods presented an average range of 0 – 26.66% more sensitive in isolating compared to the non – pellet.

From this study, *Salmonella sp.* was found to contaminate different parts of the body. This was supported by the finding (Hatha et al., 1997). That these bacteria would exist on tilapia fish's skin, gills and intestine and the most potential reservoir of salmonella spp. was the intestine. Hence, it is highly recommended that cross – contamination of other tissue notably digestive tract during handling or preparation be avoided. The whole – body of tilapia fish recorded the highest incidence as found in Aba South Market (100%). This is important for future study in order to know the route of salmonella specie transmission from pond to the next food chain supply. Hence, the pellet method and making use of appropriate media (SSA) was used to obtain more salmonella isolates in tilapia fish.

CONCLUSION

The pellet method of salmonella isolation recorded a better performance than the non-pellet method. The study showed that salmonella was more on the whole body of fish than the gills and intestine. *Salmonella shigella agar* proved to be a better selective media than the other the other two media.

REFERENCES

Abdel–Monem MH and Dowidar AA (1990). Recoveries of Salmonella from Soil in Eastern region of Saudi Arabic Kingdom, Journal Egypt Public Health Association. 65: 61–75.

Acha PN and Szyfres BC (2003). Zoonoses and communicable diseases common to man and animals. Bacterioses and mycoses. 3rd ed. Scientific and Technical Publication: 1: 580, Pan American Health Organization, Regional Office of the WHO, Washington, USA: ISBN 92 75 315809.
pp 384.

Black R, Brown K and Becker S (1984). Effects of diarrhoea associated with specific enteropathogens on the growth of children in rural Bangladish. 73: 799 – 805.

D' Aoust J, Sewell A, Daley E and Greco P (1992). Antibiotic resistance of agricultural and food borne, Salmonella isolates in Canada: 1986 – 1989. Journal Food Protection, 55: 428 – 434.

Dutch H and Altwegg M (1995). Evaluation of Five New Plating Media for Isolation of Salmonella species. Journal Clinical Microbial. 33: 802 – 304.

Esuki H, Morioka A and Ishihara K (2004). "Antimicrobial susceptibility of Salmonella Isolated from cattle, Swine and poultry (2001 – 2002): Report from the Japanese veterinary Antimicrobial Resistance Monitoring Program," Antimicrobial Chemotherapy. 53: 266 – 270.

FDA, Bacteriological Manuel Salmonella (2007). http://www.fda.gov/food/science Research/Laboratory Methods/Bacteriological Analytical Manuel BAM/UCM 070149.htm).

Heinitz ML, Ruble RD, Wagner DE and Tatini SR (2000). Incidence of Salmonella in fish and seafood. International Journal Food Microbiology. 63: 579–592.

Haltha A and Lakshmanaperumalsamy M (1997). Prevalence of Salmonella in fish and crustaceans from markets in Coimbatore, South India, Food Microbiology 14: 111–116.

Hastein T, Hjeltnes B, Lillehaug A, Utne Skare J, Berntssen M and Lundebye K (2006). Food safety hazards that occur during the production stage: challenges for fish farming and the fishing industry Review Science. Technology, 25: 607–625.

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Scienceline/Journal homepages: http://www.science-line.com/index/; http://www.ojafr.ir

Kirschner A and Velimirov B (1999). Modification of the 3 – lencincee centrifugation method for determining bacterial protein synthesis in freshwater samples. Aquatic Microbial Ecology, 17: 201–206.
Michael G, Simoneti R, de Costa M and Cardoso M (2003). Comparison of Different Selective Enrichment Steps to Isolate Salmonella Sp. from Feces of Finishing Swine Brazil Journal Microbiology. 34: 138 – 142.
Morales G, Blanco L, Arias M and Chaves C (2004). Bacteriological evaluation of fresh tilapia coming from the Northern Region of Costa Rica. Archive Latinoam Nutrition. 54: 433 – 437.
Ruiz K and Kariuki S (2004). Increasing prevalence of multidrug resistant non – typhoidal salmonella, *Kenya.* International Journal Antimicrobial Agents. 25: 38 – 43.
Thampuren N, Surendraraji A and Surendran P (2005). Prevalence and characterization of typical and atypical Escherichia coli from fish sold at retail in Cochin, India Journal Food Protection, 68: 2208 – 2211.

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