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Full Length Research Paper

Isolation of *Enterobacteriaceae* and *Pseudomonas spp*. from raw fish sold in fish market in Khartoum state

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Enterobacteriaceae **were isolated from gills, skin, muscles and the intestine of 83 out of 150 (55%) randomly collected fishes, the most dominants isolates were** ***E. coli*****,** ***Citrobacter spp*****,** ***Enteriobacter spp*** **and** ***Klebsiella spp*****. This together with the highly pathogenic** ***Enterobacteriaceae*** **including** ***Salmonella spp*** **and** ***Shigella spp*****.** ***Proteus spp*****, and** ***Alklegens*** **spp. Potential pathogenic organisms were also among the isolates. On the other hand** ***Pseudomonas spp*** **was isolated from 62% of randomly collected fishes. The number and percentages of the isolated bacteria were compared according to seasons. The total bacterial count, coliform count and** ***E. coli*** **count were estimated from all parts of collected samples. The negative impacts of the presence of** ***Enterobacteriaceae*** **and** ***Pseudomonas spp*** **in fishes were discussed based on their potential pathogenic effect toward public health and their role to enhance rapid spoilage of fishes.**

Key words: *Enterobacteriaceae*, fisheries, public health, *Pseudomonas*.

INTRODUCTION

Seafood derived from wild fish as well as farmed fish has always been an important source of protein in the human diet. 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 (Håstein et al., 2006). According to the Center for Food Safety and Applied Nutrition in Washington (2001), most fish related food borne illness are traced to *Salmonella*, *Staphylococcus spp., Escherichia spp*., *Vibrio parahemolyticus, Clostridium perfringens, Clostridium botulinum E,* and *Enteroviruses.* Thampuran et al. (2005) reported that the microbial quality of the tilapia indicated that all tissue samples except muscle tissues were contaminated with fecal coliform were *Escherichia coli* is the most common contaminant and is often encountered in high numbers. The presence of *E. coli* as well as verotoxigenic *E. coli* O157:H7 in fish meal was investigated by (Ayulo et al., 1994; Hwang et al., 2004; Thampuran et al., 2005; Ristori et al., 2007). Ristori et al. (2007) isolated *Aeromonas spp., Plesiomonas shigelloides, Vibrio cholerae* 01, *Vibrio parahaemolyticus, and Vibrio vulnificus* from different organs of fishes. It was found that the hygienic quality and freshness of fish and shellfish decreased in summer, especially for clam and mussel (Hwang et al., 2004).

Bacterial microbiota associated with fresh raw shrimp was *Aeromonas, Pseudomonas, Vibrio, Flavobacterium* and *Serratia* (Jeyasekaran et al., 2006).

Arannilewa et al. (2006) found that the total coliform count range in fish was between 3.0×10^3 - 7.5×10^6 with increasing values, as the duration of storage increases. Hood et al. (1983) found that fecal coliform levels were above the recommended wholesale level suggested by the National Shellfish Sanitation Program (less than or equal to 230/100 g). El Hadi et al. (2004) detected the presence of eight potentially pathogenic *Vibrio* species, with overall incidence in the samples as 4.6% for *V. cholerae*, 4.7% for *V. parahaemolyticus*, 6.0% for *V. vulnificus*, 11% for *Vibrio alginolyticus*, 9.9% for *Vibrio metschnikovii*, 1.3% for *Vibrio mimicus*, 13% for *Vibrio damsela*, 7.6% for *Vibrio fluvialis*, and 52% for a combined population of all of the above.

Heinitz et al. (2000) found that 10% of imported and 2.8% of domestic raw seafood was positive for *Salmonella. Enterococcus sp* and *Aeromonas sp*, fecal and total coliform, the presence of *Listeria sp* and *Salmonella spp* from the external surface of tilapias were shown by Morales et al. (2004).

Håstein et al. (2006) outlined and discussed the hazards and challenges associated with handling fish during

Table 1. Number and percentage of isolation of *Enterobacteriacaea and Pseudomonas spp* from collected 150 samples of raw fish.

Isolated organism	Positive growth		Negative growth	
	Number	Percentage	Number	Percentage
Enterobacteriacaea	83	55.3%	67	44.7%
Pseudomonas	93	62%	57	38%

Table 2. Number and percentage of *Enterobacteriacaea and Pseudomonas spp* isolated from samples collected at different seasons.

Type of organism	Winter	Summer	Autumn
Enterobacteriacaea	20 (40%)	30 (60%)	33 (66%)
Pseudomonas	32 (64%)	21 (42%)	40 (80%)
Mixed (*Enterobacteriacaea* and *Pseudomonas*)	19 (38%)	14 (28%)	17 (34%)

farming and capture and the environmental contaminants in seafood that may pose a risk to human health.

MATERIALS AND METHODS

Area of the study

This study was aimed to evaluate the hygienic quality and freshness of fresh fish (*Tilapia nilotica* Linn) and to investigate the occurrence of *Enterobacteriaceae* and *Pseudomonas spp* as indicator for fish quality. Fishes were collected randomly at 2007 from Khartoum State fish markets during the winter (January - February) and summer (March and April) and autumn (August-September) seasons. Skin surface, gills, intestinal tract and raw fish flesh were examined for each raw fish. One hundred and fifty raw fish samples were collected as 50 fish samples at each season.

Sampling

Skin: Sample from different locations of 150 raw fish of the skin was taken by rubbing the sterilized cotton swab over the skin and then inoculating into the nutrient broth.

Gills: The sterilized cotton swab was wiped against the gill filaments by lifting the operculum with the help of a pair forceps. The sample was inoculated in the nutrient broth as well as swabbed on nutrient agar. A part of the gill filament removed aseptically was also placed in a separate (nutrient broth, MacConkey broth and Selinite F broth tubes) in order to isolate all the bacteria present on the gill filaments which might have escaped contact with the swab. The examined gills were taken from 150 raw fish.

Intestine and muscles: This was done by cutting a part of intestine and muscle after sterilizing with red hot scalped and inoculation in the media (nutrient broth, MacConkey broth and Selinite F broth tubes). The samples included 150 intestines and 150 muscles from 150 raw fish.

Bacteriological examination: Preparation of the media, Isolation and identification of the bacteria were done according to Cheesbourgh (1984). Sterilization of the media was done by autoclaving at 121°C for 15 min. All samples were incubated at 37°C for 24 - 48 h.

Counting was done according to Plate count method, media used for Total bacterial count and *Pseudomonas* count (nutrient agar and Muller and Hinton), coliform count (MacConkey agar) *E. coli* count (EMB agar).

RESULTS

Isolation of organisms

Out of 150 collected samples 83 (55.3%) and 93 (62%) showed positive isolation of *Enteriobacteriaceae* and *Pseudomonas spp.* respectively. Mix isolation (isolation of both *Enterobacteriaceae* and *Pseudomonas spp* from the same sample) were recorded in 50 (40.3%) of the positive samples Table 1.

Table 2 showed that the highest isolation of both *Enteriobacteriaceae* and *Pseudomonas spp*. was recorded in autumn season as 66 and 80% respectively; this was followed by summer for *Enteriobacteriaceae* (60%) and winter for Pseudomonas *spp* (64%). The highest mixed isolation was recorded in samples collected at winter season (38%) while the highest percentage for negative isolation of both *Enteriobacteriaceae* and *Pseudomonas spp.* were shown in samples that collected at summer season (30%).

Identification of different species of isolated bacteria

The number and percentage of bacterial species which were isolated from different parts of collected fishes were shown in Table 3. Four hundred and fifty-two isolates of *Enterobacteriacaea* were recovered from all parts of collected fishes, the highest number of isolates was recovered from gills as 234 isolates (41.4%) and the lowest isolation was obtained from the muscles as 46 (8.1%).

Table 3. Number and percentage of *Enterobacteriacaea and Pseudomonas spp* isolated from 150 samples of each parts of collected fishes.

Organism	Gills	intestine	Skin	Muscles	Total No. of the isolates &%
E. coli	50 (29.2%)	33 (23.4%)	16(17%)	6 (13.0%)	105 (23.2%)
Enterobacter spp.	7 (4.1%)	4 (2.8%)	0 (0%)	0 (0%)	11 (2.4%)
Klebsiella spp.	22 (12.9%)	13 (9.2%)	9 (9.6%)	5 (10.9%)	49 (10.8%)
Citobacter spp.	8(4.7%)	12 (8.5%)	4(4.3%)	4 (8.7%)	28 (6.2%)
Salmonella spp.	2 (1.2%)	18 (12.8%)	0 (0%)	8 (17.4%)	28 (6.2%)
Shigella spp	0 (0%)	8 (2.7%)	2 (2.1%)	0 (0%)	10 (2.2%)
Alkaligenes spp	37 (21.6%)	12 (8.5%)	25 (26.6%)	14 (30.4%)	88 (19.5%)
Serritia spp	0 (0%)	0 (0%)	11 (11.7%)	0 (0%)	11 (2.4%)
Proteus spp	16 (9.4%)	11 (7.8%)	12 (12.8%)	7 (15.2%)	46 (10.2%)
Providencia spp	12 (7.8%)	23 (16.3%)	8 (8.5%)	2 (4.3%)	45 (10%)
Pseudomonas spp.	63 (55.8%)	31 (27.4)	19 (16.8%)	0 (0%)	113
Favibacterium spp	11 (6.4%)	4 (2.8%)	7 (7.4%)	0 (0%)	22 (4.9%)
Morexilla spp.	6 (3.5%)	3 (2.1%)	0 (0%)	0 (0%)	9 (2%)
Total	234 (41.4%)	172 (30.3%)	113 (20%)	46 (8.1%)	565

Table 4. Mean count of the bacteria present at different parts of examined fishes.

Collected parts	Total Bacterial Count (TBC) Cfu/ml	Coliform count Cfu/ml	*E. coli* count Cfu/ml	*Pseudomonas* count Cfu/ml
Gills	$3 \times 10^6 - 7 \times 10^9$	$3 \times 10^3 - 5 \times 10^5$	$7 \times 10^2 - 9 \times 10^4$	$5 \times 10^7 - 2 \times 10^9$
Intestine	$1.5 \times 10^5 - 1.6 \times 10^8$	$3 \times 10^3 - 7.5 \times 10^6$	$3 \times 10^2 - 4 \times 10^5$	$7 \times 10^5 - 3 \times 10^7$
Skin	$3 \times 10^7 - 4 \times 10^9$	$3 \times 10^2 - 2 \times 10^2$	$1 \times 10^2 - 7 \times 10^3$	$4 \times 10^5 - 3 \times 10^7$
Muscles	$7 \times 10^4 - 2 \times 10^6$	$1 \times 10^2 - 4 \times 10^4$	$3 \times 10^2 - 7 \times 10^3$	0

Table 3 also showed that *E. coli* was the dominant isolates as 105 isolates (23.2%) and the lowest isolates were *Shigella spp* and *Morexiella spp* with percentages a 2.2 and 2% respectively.

Pseudomonas spp, was obtained as 113 these were 63 (55.8%) isolated from gills, 31 (27.4%) isolates from the intestine and 19 (16.8%) isolates from skin. muscles samples revealed negative growth for this organisms (Table 3).

Counting of the bacteria

Table 4 showed the mean of total bacterial counts, coliform count, *E. coli* count and *Pseudomonas spp* count at different parts of collected fishes, the gills showed the highest number of bacteria; this was followed by skin then muscles.

DISCUSSION

Fishery products are important not only from a nutritional point of view, but also as an item of international trade and foreign exchange earner for a number of countries in the world. Fish and shellfish are highly perishable, and prone to vast variations in quality due to differences in species, environmental habitats, feeding habits. In addition, they can also function as carriers of several microbial and other health hazards. Therefore maintenance of quality is of utmost importance in production and trade of fishery products. Most of current quality control techniques are time consuming and cumbersome.

Although only a few infectious agents in fish are able to infect humans, some exceptions exist that may result in fatalities. However, the greatest risk to human health is due to the consumption of raw or insufficiently processed fish and fish products. In this study isolation of Enterobacteriacaea as 55% from collected samples indicated public health hazards and concern, particularly isolation of some highly pathogenic agents such as Salmonella *spp., Shigella spp*., and the pathogenic *E. coli*. and potential pathogenic organisms such as *Klebsiella spp., Citrobacter spp., Proteus spp.* The isolation of these groups of organisms indicated faecal and environmental pollution and these supported the findings of Yagoub et al. (2004) who isolated pathogenic and potential pathogenic organisms from tap water that originated from Nile river. This also confirms the findings of Koutsoumanis and Nychas (2000); Gonzalez-Podriguez et al. (2001) and

Herrera et al. (2006) who isolated similar organisms from fish and fish products. The isolation of *Alkaligenes* which are widely distributed in soil, water and intestinal tract of vertebrates and play an important role in the spoilage of fish and fish meat support the findings of Collins et al. (1989).

The isolation of *Pseudomonas spp* from 63% of collected fish samples is of highly importance because this bacterium plays a considerable role as potential pathogenic bacteria for human and as an indicator of food quality as spoilage organism. This is in accord with previously mentioned by Jeyasekaran et al. (2006) and Koutsoumanis and Nychas (2000) who identified *pseudomonads* as a good spoilage index.

The highest isolation of microorganisms during the autumn season might be due to rain drainage that carries polluted soil to river water when the presence of high organic substances promoted multiplication of the organisms. The high percentage of the presence of Enterobacteriacaea in summer and *Pseudomonas spp* in winter season might be partially attributed to the suitability of the temperature for the survival and multiplication of these bacteria.

This study revealed that raw fish sold in fish market in Khartoum state could be a source of food- borne bacterial pathogens. Improvements in handling and processing are needed to minimize the prevalence of the pathogenic bacteria. The total viable *Enterobacteriaceae* counts strongly suggest the urgent need to improve the quality control systems. The present results may be considered as additional knowledge to enhance proper controlling of the storage life of fish, and fish product quality.

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