

Original Research Article

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Bacteriological Study of Fish Samples Collected from Different Markets in Some Egyptian Governorates and Antimicrobial Sensitivity of Isolates

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ABSTRACT

A total of (80) samples of living diseased cultured Tilapia fish (*Oreochromis niloticus*), were collected from different fish farms in Egypt (Behera and Kafr El-Sheikh) which showed the clinical signs of loss of scales from some areas of the skin, excessive mucus all over the body surfaces with petechial haemorrhages over the dorsal musculature, large necrotic lesions extending all over the body and darkness of skin. Differentiation and characterization of various isolates was based on their growth characteristics on specific culture media (biochemical and gram staining reactions). The following human pathogenic bacteria were isolated *Staph. aureus*, *Staph. epidermidis*, *Staph. Saprophytics*, *Streptococcus spp.*, *E. coli*, *Salmonella*, *P. aeruginosa*, *P. fluorescens*, and Enterobacteriaceae from skin with the incidence of 12.5%, 23.8%, 31.3%, 10%, 25%, 7.5%, 22.5%, 20%, and 18.8% respectively, but from muscle with the incidence of 7.5%, 7.5%, 12.5%, 8.8%, 22.5%, 5%, 20%, 18.8%, and 16.3% respectively. On the other hand these bacteria isolated from intestine with the incidence of 8.8%, 7.5%, 12.5%, 13.8%, 25%, 8.8%, 17.5%, 15%, and 16.3% respectively, while from liver with incidence of 15%, 12.5%, 16.3%, 15%, 35%, 6.3%, 25%, 23.8%, and 15% respectively. In vitro sensitivity test indicated that, the most prevalent bacteria isolated from examined fish samples were sensitive to enrofloxacin, norfloxacin, ciprofloxacin and kanamycin. Most of these strains were highly resistant to erythromycin and a moxycillin. PCR panel could help for rapid diagnoses to determine the causative agents from fish samples, *Staph. aureus* coagulase gene and variable fragments for 16SrRNA genes from the extracted DNA at 228bp.

Keywords

Haemorrhages,
Fluorescens,
Norfloxacin,
Bacteria,
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Introduction

Fish and fish products have long been used as a major food component for humans and animals. Fishes are known to be enriched by high nutritional components and concentrated source of energy, in addition to their high palatability and good digestibility (Mead *et al.*, 1986; Mol *et al.*, 2007; Dinakaran *et al.*, 2010; Kawarazuka, 2010).

Fish is a perishable protein food, when fish is stored at 10°, it remains for about 40 hours

before it begins to spoil. Freezing does not prevent spoilage of fish because of autolytic activities and chemical changes occurring in fish after harvest (Huss *et al.*, 1974, and Jay Jm modern food microbiology, 1992).

The degeneration of fish is accelerated by microorganism associated with aquatic environment as well as contaminated during post –harvest handling, when fish dies microorganisms on the surface as well as gut

and gills begins to utilize the fish protein and food nutrient resulting in loss of nutritional value (Ames,1992). Microbial activities create undesirable changes like off-flavors, texture and appearance (Jhonstone *et al.*, 1994).

Fish disease due to bacterial infection are considered one of the major problems in aquaculture which lead to heavy losses (Austin and Allen Austin 1985 and Austin and Austin, 1999), causing a great drop in fish production and industry. Most of the bacteria associated with these diseases are naturally saprophytic organism and widely distributed in the aquatic environment (Frerichs and Handrie, 1985).

A variety of fishes consumed regularly are prone to pathogenic spoilage especially by *Vibrio spp.*, *Shigella spp.*, *Salmonella spp.*, *streptococci*, *Staphylococci*, *Coliforms*, *Listeria spp.*, *Clostridium spp.* (Rahman *et al.*, 2012) which may get entry into the fish from their habitat or during the fish transportation and storage (Frazier and Westhoff, 1995; Eze *et al.*, 2010). A number of reports suggested that the consumption of the microbiologically spoiled seafoods might be responsible for food-borne diseases like diarrhea, salmonellosis, shigellosis, cholera and even some neurological diseases by an array of viruses, bacteria, fungi and parasites (Snowdon *et al.*, 1989; Starutch, 1991; Karunasagar *et al.*, 1994; Cray and Moon, 1995; Wallace *et al.*, 1999; WHO, 2012).

However fish are susceptible to a wide variety of bacterial pathogens, most of which are capable of causing disease and are considered by some to be saprophytic in nature (Lipp and Ross, 1997). The microbiological diversity of fresh fish muscle depends on the fishing grounds and environmental factors around it (Cahill, 1990). It has been suggested that the type of micro-organisms that are found

associated with particular fish depends on its habitat (Claucas and Ward, 1996). The bacterial pathogens associated with fish have been classified as indigenous and non-indigenous (Kvenberg, 1991). The non-indigenous contaminate the fish or the habitat one way or the other and examples include *Escherichia coli*, *Clostridium botulinum*, *Shigella dysenteriae*, *Staphylococcus aureus*, *Listeria monocytogens* and *Salmonella*. The indigenous bacterial pathogens are found naturally living in the fish s habitat for example *Vibrio* species and *Aeromonas* species (Rodricks, 1991). The bacteria from fish only become pathogens when fish are physiologically unbalanced, nutritionally deficient, or there are other stressors, i.e., poor water quality, overstocking, which allow opportunistic bacterial infections to prevail (Austin, 2011). Pathogenic and potentially pathogenic bacteria associated with fish and shellfish include *Mycobacterium*, *Streptococcus spp.*, *Vibrio spp.*, *Aeromonas spp.*, *Salmonella spp.* and others (Lipp and Ross, 1997).

Staphylococcus, *Escherichia coli*, *Pseudomonas*, *Shigella* and *Salmonella*, were the common pathogenic bacteria found associated with fish from the ponds associated with integrated farming systems. Their presence was attributed to the contamination of the fish ponds by animal waste (Abdelhamid *et al.*, 2006). The isolation of *Salmonella*, *Shigella* and *E. coli* from the fish samples indicates faecal contamination of the ponds resulting from the livestock manure that they add to the fish ponds as feed. The isolation of *Salmonella*, *Shigella*, and *E. coli* indicate faecal and environmental pollution (Yagoub, 2009). Coliforms such as *E. coli* are usually present where there has been faecal contamination from warm blooded animals (Chao *et al.*, 2003). *E. coli* is recognized as the reliable indicator of faecal contamination in small numbers and in large numbers, it is

an indicator of mishandling (Eze *et al.*, 2011). *E. coli* is the only species in the coliform group that is found in the human intestinal tract and in the other warm blooded animals as a commensal and is subsequently excreted in large quantities in faeces (Geldreich, 1983).

One of the risks involved in livestock integrated fish farming is possible transfer of pathogens between livestock and humans. Previous research has shown that, different kinds of livestock manure are contaminated with pathogenic bacteria such as *Salmonella*, *Shigella*, *Pseudomonas*, *Vibrio*, *Streptococcus*, and *E. coli* species (Abdelhamid *et al.*, 2006). Rate of bacterial spoilage is dependent on the initial microbial load, ambient temperature and improper handling. Therefore, proper storage critical in maintaining a high standard of safety when processing fish. (Jay Jm modern food microbiology, 1992).

The transmission of these pathogens to people can be through improperly cooked food or the handling of the fish. There have been great economic losses reported due to food borne illness such as dysentery and diarrhea resulting from consumption of contaminated fish and such can be a problem to the immune compromised, children and elderly people.

The aim of the present study is to prove the demonstration, isolation and identification of of human pathogenic bacteria from the skin, muscle, intestines and liver of living diseased fish samples.

Besides, susceptibility of most predominant isolates to chemotherapeutic agents as an aid to overcome this problem and reduce losses. Also, using polymerase chain reaction (PCR) test to substitute the conventional cultural methods and rapid diagnosis of *Staph. aureus* directly from fish samples.

Materials and Methods

Samples

A total of (80) samples of living diseased Tilapia fish (*Oreochromus niloticus*), were collected from different fish farms in Egypt (Behera and Kafr El-Sheikh), during the period from May 2014 till February 2015. The collected fish subjected to gross clinical examination according to the method described by (Amlacher 1970), showed the clinical signs of Loss of scales from some areas of the skin, excessive mucus all over the body surfaces with petechial haemorrhages over the dorsal musculature. Large necrotic lesions extending all over the body with tail rot. Skin showed superficial ulcers, and darkness of skin (Fig. 1). The fish samples were transferred aseptically to laboratory of microbiology without delay in large sterile jars containing water.

The surface of fish bodies were disinfected by alcohol (70%), then dissected under antiseptic conditions. The freshly dead collected samples of fishes were subjected to post mortem examination (PM) before the bacteriological examination according to (Lucky 1977, and Conroy and Herman 1981). The post mortem examination revealed of generalized septicemia and enlargement in most internal organs especially kidney, liver, intestinal tract, spleen and severe distension of gall bladder with bile secretion, congested gills and gall bladder (Fig. 2).

Bacteriological examination

Specimens of fish obtained from skin, muscle, intestine and liver were inoculated directly in nutrient broth and incubated at 37°C for 6 hours. Loopful from each broth culture were inoculated directly onto nutrient agar, blood agar, Macconkey's agar, bile salt lactose agar, Salmonella. Shigella agar, Mannitol Salt agar,

Baird parker's agar media for isolation of *S. aureus*, Eosin Methylene blue agar media for isolation of *E. coli* and Enterobacteriaceae family. The plates were incubated at 37°C for 24-48 hours. Suspected colonies onto the surface of these media were identified by studying characters of the colonies as well as Gram's stain, then identified morphologically according to the method described by (Kloss and Schleifer 1986, Barrow and Feltham 1993 and Austin and Austin 1999).

One single colony showed typical colonial appearance and morphological characters was picked up and streaked into semisolid agar media and incubated at 37°C for 24 hours to obtain pure culture, for further identification. The pure colonies were biochemically identified according to (Cruickshank *et al.*, 1975, Koneman *et al.*, 1992, Quinn *et al.*, 2002). The Gram negative bacteria included Enterobacteriaceae family were biochemically identified according to (Morrison *et al.*, 1981 and Krieg and Holt 1984).

Antibiotic sensitivity tests (Antibiogram)

The sensitivity of bacterial isolates to different antimicrobial agents were investigated using the disc diffusion method as described by (Lennette *et al.*, 1980, and Finegold and Martin 1982) to detect the drug of choice against different isolated bacteria for trials of treatment. The results were interpreted according to (Koneman *et al.*, 1992).

Extraction of *Staph. aureus* DNA (Løvseth and BerdalK 2004)

Isolated *Staph. aureus* strains were incubated overnight in 10 ml brain heart infusion broth (Oxoid), centrifuged at (5000 rpm, for 15 min) and resuspended in 0.5 ml TE buffer (10 mM Tris, 1 mM EDTA - pH 8). Total cellular DNA was extracted using Qiagen DNA

extraction kit (Qiagen, Germany) according to manufacturer's protocol for gram-positive bacteria. The extracted DNA from samples was dissolved in 25 µl sterile distilled water and stored at -20°C until further use.

Multiplex PCR was performed on the extracted DNA from samples (part C) to detect coagulase (*coa*) and 16SrRNA genes (Hookey *et al.*, 1998 and Løvseth and BerdalK 2004).

Primers for *Staph. aureus* coagulase (*coa*) and 16SrRNA genes

Specific oligonucleotide multiplex primer assay (synthesized by MWG-Biotech AG, Holle & Huttner GmbH, Germany), for rapid diagnosis of *Staph. aureus* coagulase (*coa*) and 16SrRNA genes. The forward primer for coagulase (*coa*) was 5'-ATAGAGATGCTGGT -3', while the reverse primer was 5'-GCTTCCGATTGTTCG -3' (Hookey *et al.*, 1998). While the forward primer for 16SrRNA gene was 5'-GTAGGTGGCAAGCG -3', while the reverse primer was 5'-CGCACATCAGCGTC -3' (Løvseth and BerdalK 2004).

***Staph. aureus* DNA amplification by PCR**

The PCR was performed (Hookey *et al.*, 1998 and Løvseth and BerdalK 2004) in a touchdown thermocycler in a total reaction volume of 30 µl containing 2.5 µl of extracted DNA, 1 µl of each primer (10 pmol/µl), 0.6 µl of deoxynucleoside triphosphate (10 mmol/L), 3 µl of 10 X thermophilic buffer (Promega), 1.8 µl of MgCl₂ (25 mmol/L), 0.1 µl of Taq DNA polymerase (5 U/µl), and complete the reaction volume using distilled water in 0.2-ml reaction tube. The presence of PCR products was determined by electrophoresis of 10 µl of the DNA product in a 1.5 % agarose gel with 1 X TAE buffer (40 mM Tris-HCl, 1 mM EDTA/L, 1.14 ml/L

glacial acetic acid, pH 7.8) at a voltage of 4 volts /cm and stained with 0.5 mg/ml ethidium bromide and the Fluorescent bands were visualized with a UV transilluminator and photographed. A 100-bp DNA ladder (Gibco BRL) was used as a molecular marker. Amplification was obtained with 35 cycles. Each cycle involved initial denaturation at 93°C for 3 minutes, denaturation at 92°C for 1 minutes, annealing at 52°C for 1 minutes, and extension at 72°C for 1 minutes. The final extension was performed at 72°C for 7 minutes.

The presence of PCR products was determined by electrophoresis of 10 µl of the DNA product in a 1.5 % agarose gel with 1 X TAE buffer (40 mM Tris-HCl, 1 mM EDTA/L, 1.14 ml/L glacial acetic acid, pH 7.8) at a voltage of 4 volts /cm and stained with 0.5 mg/ml ethidium bromide and the Fluorescent bands were visualized with a UV transilluminator and photographed. A 100-bp DNA ladder (Gibco BRL) was used as a molecular marker.

Results and Discussion

Results in table 1 shows the total bacterial isolates from different site of (80 skin, 80 muscle, 80 intestine and 80 liver) of examined naturally infected fishes which were (30.4%, 21.1%, 22.2 % and 26.2 % respectively). The prevalence of bacterial isolates from the different examined naturally infected fishes is illustrated in table 1 also. The result revealed that isolation rate was (10.9) for *Staph. aureus*, (12.8) for *Staph. epidermidis*, (18.1) for *Staph. Saprophytics*, (11.9) for *Streptococcus spp.*, (22.8) for *E. coli*, (6.9) for *Salmonella*, (21.3) for *P. aeruginosa*, (19.4) for *P. fluorescens* and (16.6) for Enterobacteriaceae.

Table 2 showed the in vitro sensitivity of the most prevalent bacteria isolated from collected fish samples were done against (14)

chemotherapeutic agents. Most tested strains of *Staph. aureus*, *Staph. epidermidis*, *Staph. Saprophytics*, *Streptococcus spp.*, *E. coli*, *Salmonella*, *P. aeruginosa*, *P. fluorescens*, and Enterobacteriaceae were sensitive to enrofloxacin, norfloxacin, ciprofloxacin and kanamycin. Most of these strains were highly resistant to erythromycin and amoxicillin.

Figure 3 showed three diseased fish samples representative for positive *Staph. aureus* isolates, were selected and subjected to PCR analysis. The specificity of the oligonucleotide primer was confirmed by the positive amplification of 228bp fragments for *Staph. aureus* coagulase (coa) and variable fragments for 16SrRNA genes from the extracted DNA of *Staph. aureus*.

Aquaculture products can harbor pathogenic bacteria which are part of the natural microflora of the environment. Bacterial pathogens associated with fish can be transmitted to human beings from fish used as food or by handling the fish causing human diseases. The role of bacteria varies from their effect as primary pathogen to that of secondary invader in the presence of other disease agents; they may also serve as a stress factor and predispose fish to other diseases (Badran and Eissa, 1991).

The isolation of enteric bacteria in fish serves as indicator organisms of faecal contamination and or water pollution. Their presence also represents a potential hazard to humans. Generally, the presence of coliform and faecal coliform is not the normal flora of bacteria in fish (Mandal *et al.*, 2009). This is reflecting the contamination of fish habitat with the human and animal faeces.

Staphylococcus spp. It is associated with food poisoning, produced toxin, which makes man sick, usually associated with the nausea, vomiting and diarrhea after eating the staphylococci infected food (O'connell, 2002).

In the present work, we spot light on the clinical picture and PM lesions of the most predominant bacterial pathogens affecting *Oreochromus niloticus* fishes. Moreover, isolation and identification of these bacterial infections by both biochemical traditional methods serological as well as by PCR, Concerning the clinical signs and Postmortem (PM) lesions of examined *Oreochromus*

niloticus fish. In regard to the results of clinical signs and PM lesions of bacterial naturally infected fishes, our results were similar to the findings recorded by (Post 1987, Badran and Eissa, 1991 and Austin and Austin, 1999), where they mentioned that bacterial infection causes generalized septicemia and enlargement in most internal organs.

Table.1 Prevalence of bacteria isolated from skin, muscle, intestine and liver of collected fish samples

<i>Bacterial isolates</i>	Site of isolation									
	Skin (80)		Muscle(80)		Intestine(80)		Liver(80)		Total	
	No.	%*	No.	%*	No.	%*	No	%*	No	%**
<i>Staph. aureus</i>	10	12.5	6	7.5	7	8.8	12	15	35	10.9
<i>Staph. epidermidis</i>	19	23.8	6	7.5	6	7.5	10	12.5	41	12.8
<i>Staph. saprophytics</i>	25	31.3	10	12.5	10	12.5	13	16.3	58	18.1
<i>Streptococcus spp.</i>	8	10	7	8.8	11	13.8	12	15	38	11.9
<i>E. coli</i>	20	25	18	22.5	20	25	15	35	73	22.8
<i>Salmonella</i>	6	7.5	4	5	7	8.8	5	6.3	22	6.9
<i>P. aeruginosa</i>	18	22.5	16	20	14	17.5	20	25	68	21.3
<i>P. fluorescens</i>	16	20	15	18.8	12	15	19	23.8	62	19.4
<i>Enterobacteriaceae</i>	15	18.8	13	16.3	13	16.3	12	15	53	16.6
Total bacterial isolates	137	30.4	95	21.1	100	22.2	11	26.2	45	100

*% was calculated according to total number of samples (80)

**% was calculated according to the total number of samples (320)

Fig.1 *Oreochromus niloticus* naturally infected with bacteria showing petechial haemorrhages over the dorsal musculature. Large necrotic lesions extending all over the body



Table.2 Results of antibiogram pattern of the most prevalent bacteria Isolated from collected fish samples

Antibacterial agents	<i>Staph. aureus</i>		<i>Staph. epidermidis</i>		<i>Staph. Saprophyticus</i>		Streptococcus		<i>E.coli</i>		<i>Salmonella</i>		<i>P. aeruginosa</i>		<i>P. fluorescens</i>		<i>Enterobacteriaceae</i>		
	S.	%	S.	%	S.	%	S.	%	S.	%	S.	%	S.	%	S.	%	S.	%	
Amoxicillin (25ug)	6/15	40	5/15	33.3	2/15	13.3	6/15	40	0/15	0	2/15	13.3	1/15	6.7	2/15	13.3	1/15	6.7	
Chloramphenicol (30ug)	6/15	40	5/15	33.3	7/15	46.7	11/15	73.3	0/15	0	9/15	60	9/15	60	10/15	66.7	2/15	13.3	
Ciprofloxacin (5ug)	14/15	93.3	13/15	86.7	14/15	93.3	13/15	86.7	14/15	93.3	14/15	93.3	15/15	100	15/15	100	13/15	86.7	
Erythromycin (10ug)	1/15	6.7	1/15	6.7	5/15	33.3	3/15	20	2/15	13.3	2/15	13.3	0/15	0	1/15	6.7	3/15	20	
Flumequine (30ug)	11/15	73.3	8/15	53.3	14/15	93.3	11/15	73.3	1/15	6.7	1/15	6.7	0/15	0	1/15	6.7	14/15	93.3	
Gentamicin (10ug)	10/15	66.7	15/15	100	14/15	93.3	14/15	93.3	13/15	86.7	13/15	86.7	12/15	80	13/15	86.7	9/15	60	
Norfloxacin (10ug)	15/15	100	13/15	86.7	12/15	80	15/15	100	15/15	100	15/15	100	8/15	53.3	2/15	13.3	13/15	86.7	
Polymyxin (10ug)	9/15	60	12/15	80	6/15	40	8/15	53.3	1/15	6.7	1/15	6.7	0/15	0	2/15	13.3	6/15	40	
Streptomycin (10ug)	12/15	80	10/15	66.7	8/15	53.3	5/15	33.3	14/15	93.3	14/15	93.3	9/15	60	10/15	66.7	5/15	33.3	
Penicillin G (10ug)	0/15	0	13/15	86.7	7/15	46.7	9/15	60	1/15	6.7	1/15	6.7	0/15	0	1/15	6.7	5/15	33.3	
Kanamycin (30ug)	13/15	86.7	12/15	80	13/15	86.7	13/15	86.7	15/15	100	15/15	100	13/15	86.7	100	14/15	93.3	15/15	100

S: Sensitive; %: Percentage of sensitive isolates in relation to total isolates.

Fig.2 *Oreochromis niloticus* naturally infected with bacteria showing generalized septicemia and enlargement in most internal organs

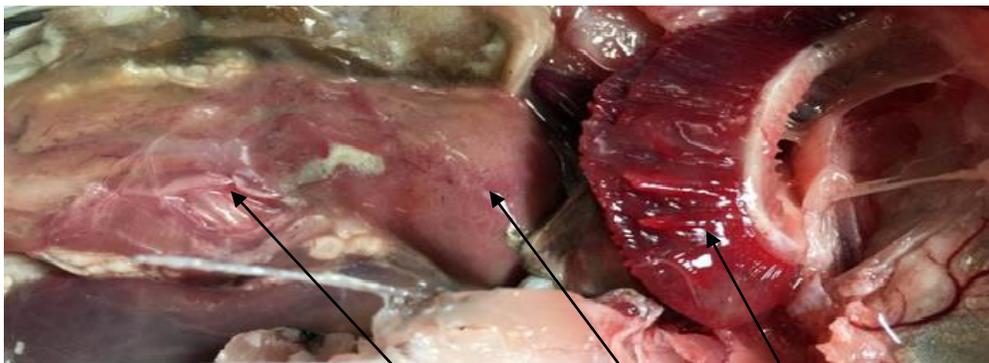
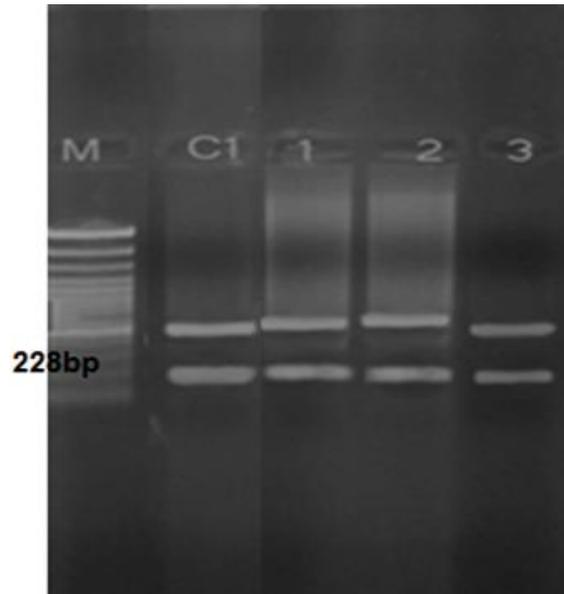


Fig.3 Electrophoresis analysis of PCR product of amplified *Staph. aureus* coagulase (coa) and 16SrRNA genes



M: 100bp marker.

Lane 1, 2 and 3 indicate a positive amplification *Staph. aureus* coagulase (coa) at the 228bp and variable for 16SrRNA genes:

C1: Control positive for *Staph. aureus* coagulase (coa) and 16SrRNA genes

In regards to the Incidence of the bacterial isolates among naturally examined fishes as shown in table 1, the most predominant isolates from skin were *Staph. Saprophytics* (31.3%), *E. coli* (25%), *Staph. Epidermidis* (23.8%), *P. aeruginosa* (22.5%), *P. fluorescens* (20%), Enterobacteriaceae (18.8%), *Staph. aureus*, (12.5%), *Streptococcus spp.* (10%) and *Salmonella* (7.5%), while the most predominant isolates from muscle were *E. coli* (22.5%), *P. aeruginosa* (20%), *P. fluorescens* (18.8%), Enterobacteriaceae (16.3%), *Staph. Saprophytics* (12.5%), *Streptococcus spp* (8.8%), *Staph. aureus* (7.5%), *Staph. Epidermidis* (7.5%) and *Salmonella* (5%). On the other hand the most predominant isolates from intestine were *E. coli* (25%), *P. aeruginosa* (17.5%), Enterobacteriaceae (16.3%), *P. fluorescens* (15%), *Streptococcus spp.* (13.8%), *Staph. saprophytics* (12.5%), *Staph. aureus* (8.8%), *Salmonella* (8.8%) and *Staph. epidermidis* (7.5%), but the most predominant isolates from liver were *E. coli*

(35%), *P. aeruginosa* (25%), *P. fluorescens* (23.8%), *Staph. saprophytics* (16.3%), *Staph. aureus* (15%), *Streptococcus spp.* (15%), Enterobacteriaceae (15%), *Staph. epidermidis* (12.5%) and *Salmonella* (6.3%).

In similar studies, *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Salmonella typhi* were isolated from the gills, intestines, muscle and skin of *Megalaspiscordyla* and muscles of *Priacanthushamrur* from Royapuram waters in India by (Sujatha *et al.*, 2011). This was attributed to the heavy load of sewage disposal into the seas which could act as a suitable environment for the growth and survival of the human pathogens. Members of the genus *Pseudomonas* are found in the soil and natural sources of water and are important phytopathogens and agents of human infections being considered opportunistic pathogens (Sujatha *et al.*, 2011).

This also confirms the findings of (Koutsoumanis and Nychas, 2000; Gonzalez-Podriguez *et al.*, 2001; Herrera *et al.*, 2006), who isolated similar organisms from fish and fish products. The presence of these bacterial species in fish has been reported to be of public health significance, because of their primary role as occupational hazard to fish handlers (Ibiwoye *et al.*, 2001).

Table 2 showed the in vitro sensitivity of the most prevalent bacteria isolated from collected fish samples were done against (11) chemotherapeutic agents. Most tested strains of *Staph. aureus*, *Staph. epidermidis*, *Staph. saprophyticus*, *Streptococcus spp.*, *E. coli*, *Salmonella*, *P. aeruginosa*, *P. fluorescens* and Enterobacteriaceae were sensitive to enrofloxacin, norfloxacin, ciprofloxacin and kanamycin. Most of these strains were highly resistant to erythromycin and amoxicillin. The data generated from this experiment are essential in the choice of most effective antimicrobial agents against fish pathogenic bacteria of fish. This results nearly agreed with those of (Zaky 2009) who arranged the antibiotic according its effect on *E. coli* isolated from fish, the least to high were chloramphenicol (30 mcg), ampicillin (10 mcg), penicillin G (10 mcg), streptomycin (10 mcg) and Gentamycin (10 mcg).

Antimicrobial agents are widely used by farmers especially in the intensive culture system. Misuse of drugs and non-compliance with treatment regimens among users can cause treatment to be less effective thereby prolonging the duration of disease (Muniruzzaman and Choudhury, 2004).

The use of polymerase chain reaction (PCR), as shown in figure 3, revealed positive amplification of *Staph. aureus* on 228 bp fragments for *Staph. aureus* coagulase (coa) and variable fragments for 16SrRNA genes from the extracted DNA of *Staph. aureus* on lane 1-3. These results suggest that the PCR assay could be used as an alternative method in routine diagnosis for rapid, sensitive, and specific

simultaneous detection of *Staph. aureus* in fish samples. Our results were in agreement with those of other studies (Coton and Coton, 2005; Janda and Abbott, 2007; Olivares *et al.*, 2008). Also, these results are agreement with the results of (Phuektes and Browning, 2001; Gillespie and Oliver, 2005; Cremonesi *et al.*, 2006).

In conclusion, fish are susceptible to all contaminant organisms that may be found in water, post harvesting, marketing, dealing, fish handlers, this processing will result in microbiological activities leading to loss of fish meat quality so the basic principles for prevention of food borne disease and sanitation should be followed to protect the consumers against the public health hazard, the widespread presence of antibiotic resistance microorganisms should be a priority to reinforce the importance of basic hygiene for fish.

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