



RESEARCH ARTICLE

Isolation of bacteria from fish

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Abstract

This study carried out to isolation of bacteria from fish.(100) rectal swab from fish were collected from different area of local markets in Baghdad city (28) sample from Ilisha (1) sample from *B. arbus xanthopterus* ,(1) sample from *Barbus grypus* ,(24) sample from *cyprinu carpio* ,(25) sample from *liza abu*,(4) sample from *aspilus varax* ,(7) sample from *barbus luteus* ,(2) sample from *barbus sherppei* ,(1) sample from *carrarius auratus* ,(2) sample from *mugil cephalus* ,(5) sample from *hypophthalmichthys molitrix* . samples culturing on different media upon cultural , morphological and biochemical examination. The result revealed that isolation rate was (49%) for *Staphylococcus aureus* , (49%)for *Streptococcus* group D,(39%) for *Salmonella* spp ,(27%)for *Proteus* spp (15%) for *Pseudomonas* spp, (15%) for *Escherichia coli* ,(15%) *Aeromonas* spp,(26%) *Vibrio parahaemolyticus*, (3%) *Plesiomonas shigelloides* , (3%) *Citrobacter* , (12%) *Edwardsiella* and (4%) *Nocardia*.

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Introduction

Fish is a perishable protein food, when fish is stored at $<10^{\circ}$, it remains for about 40 hours before it begins to spoil. (1, 2) Freezing does not prevent spoilage of fish because of autolytic activities and chemical changes occurring in fish after harvest. (1, 2) 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(3) Microbial activities create undesirable changes like off-flavors, texture and appearance (4) 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.(2). Hook fishing for instance keeps the earlier caught fish waiting, often UN gutted and with no ice while the fisherman tries to accumulate a good number to trade(5). In net fishing, the net are laid overnight or for long hours. in such cases, if fish dies under water with high ambient temperature (water temperature $18-21^{\circ}$) the spoilage begins right under water (6).

Poor sanitation and hygiene lead to cross contamination and multiplication of microorganisms and hence poor quality of fish are presented to costumers (7)

Studies indicate that there are several bacteria that have been isolated from three main parts of fresh fish, slime layer, gills and the gastrointestinal tract (8)

The microbiological diversity of the fresh fish muscle depends on the fishing grounds and environmental factors around it, fish from warm waters mostly harbor mesophilic gram negative bacteria while cold waters harbor mostly psychrophilic gram positive bacteria, these bacterial isolates are classified into two groups: Endogenous and post-harvest bacteria. Previous studies have also demonstrated the presence of indicator microorganisms of fecal pollution, opportunistic and pathogenic bacteria to human in fish (8, 9, 10, 11, and 12)

The consequences of fish spoilage are far reaching and more than just the loss of protein. There have been great economic losses reported due to food borne illness as the result of consuming contaminated fish, the microbial association with fish compromises safety and the quality for human consumption, particularly critical is when the microorganisms are opportunistic and pathogenic in nature (13).

Considering the problems relating to poor handling and insufficient and improper storage facilities on the streets, the risks of contracting food borne disease by consumers may be high, the circumstances and the growing demands for fish prompted this research to look into the safety and quality of fish sold at supermarkets (14)

Seafood derived from wild fish as well as farmed fish has always been important source of protein in the human diets, on the global scale fish and fish product are the most important source of protein and it is estimated that more than 30% of fish for human consumption comes from aqua culture (15)

Materials and methods

Collection of samples: 100 rectal swab were collected from 100 fish: (28) swab from ilisha, (1) swab from barbus xanthoptereus, (1) swab from barbus grypus, (24) swab from cyprinu carpio, (25) swab from Liza abu, (4) swab from Aspilus vorax, (7) swab from barbus luteus, (2) swab from barbus sherpeyi, (1) swab from carraius auratus, (2) swab from mugil cephitus, (5) swab from hypophthalmichthys molitrix. Samples of fish were randomly collected from different markets of Baghdad city, sample transported to the laboratory within half hour and kept in the refrigerator at (4⁰) cultures to bacteria were done within (2-4) hours of collection of samples. All swabs were then inoculated on maconkey agar. Salmonella , shigella, (SS)agar media and manitol salt agar, kanamycin agar, TCBS agar Eosin methylene blue agar.

Suspected pathogens were further identified by growth characters colony morphology, gram staining, motility, different biochemical reaction using the procedure outlined by (14).

RESULT & DISCUSSION

The result revealed that isolation rate was:

49% Staphylococcus aureus., 49% Streptococcus group D, 39% Salmonella spp., 27% Proteus spp., 15% Pseudomonas spp., 26% Vibrio spp., 15% Escherichia coli, 15% Aeromonas spp. plesiomonas shigelloides 3%, citrobacter 12%, Edwardsiella 1%, Nocardia spp 4 %.

Fish are an important supplement of human diet in many parts of the world. In this study different type of bacteria of human importance were isolated from different species of fish collected from different market of Baghdad city. Most fish related food borne illnesses are traced to salmonella, staphylococcus spp., Escherichia spp., Vibrio Parahaemolyticus, Clostridium Perfringens, Clostridium botulinum and enteroviruses (16)

The presence of E.coli as well as neurotoxicogenic E.coli O157:H7 in fish meal was investigated by (17, 18, 19, and 20).

(20) Isolated Aeromonas spp., Plesiomonas shigelloides, Vibrio cholerae 01, and Vibrio parahaemolyticus and Vibrio vulnificus from different organs of fish, it was found that the hygienic quality & freshness of fish and shell fish decreased in summer especially for clam and mussel (18)

(21) found that the total coli form count range in fish was between 3.0×10^3 - 7.5×10^5 with increasing values. As the duration of storage increases.

(22) found that fecal coli forms levels were above the recommended whole sale level suggested by the national shell fish sanitation program (less than or equal to 230/100 g). (23) detected the presence of eight potentially pathogenic Vibrio species, with overall incidence in the samples as 4.6% for Vibrio cholera, 4.7% for Vibrio Parahaemolyticus, 6.0% for Vibrio vulnificus, 11% for Vibrio alginolyticus, 9.9% for Vibrio metschnikowii, 1.3% for Vibrio mimicus, 13% for Vibrio damsela, 7.6% for Vibrio fluvialis, 52% for a combined population of all of above.

(24) Found that 10% of important and 2.8% of domestic raw seafood was positive for Salmonella, Enterococcus spp., and Aeromonas spp. fecal and total coli form, the presence of Listeria spp. And Salmonella spp. From the external surface of tilapias were by (25). (15) Outlined and discussed the hazards and challenges associated with handling fish during farming and capture and the environmental contaminants in seafood that may pose a risk to human health.

The presence of Escherichia coli is currently used as an indicator of recent fecal contamination in recreational waters, they are many possible sources of E.coli in the environment including waterfowl (26,27,28), wild life (all animals except waterfowl) (27,29,30) algae and periphyton (31,32,33)

Soils and sediments(34,35) and treated wastewater effluent(27) wild fish, are relatively understudied and often an overlooked potential source of E.coli. there have been several studies on the presence of fecal coli forms(FC) in farm-reared fish because of concern about the health of fish consumers.

(36)reported that bacteria in general Aeromonas, burkholderia, chromo bacterium citrobacter, Escherichia, Flavimonae, and Plesiomonas are present in farm-raised tilapia.

(37) Used E.coli-laden feed to infect rainbow trout (*onchorhynchus mykiss*) intestine.

(38) Detected E.coli in farm raised tilapia intestines and correlated them with pigeon droppings. (39) Found two fish species that harbored E.coli from a river contaminated by sewage effluent, their work also supported the view that fish obtain E.coli from the environment. (40) Which have been used to identify the source of environmental E.coli can also be used to identify the sources of E.coli in fish. Knowing these sources would allow us to better understand the role, if any that fish play in microbial contamination of aquatic habitats.

Fish are susceptible to a wide variety of bacterial pathogens especially when the fish are physiologically unbalanced or nutritionally deficient or subjected to stress or i.e. poor water quality and overstocking. infectious diseases are the main cause of economic losses in aquaculture industry which is negatively impacted by various pathogenic organisms (41). Such as *Edwardsiella tarda* (gram negative enterobacterium) which is the causative agent of *Edwardsiellosis* in freshwater (42)

Over-exploitation of fish for non-food use is now causing concern, particularly for some of the species used as traditional medicines.

Vibrio has been isolated from diseased marine fish such as (*acanthopagrus cuvieri*) sea bream ,*Sparusaurata* (43)

It has been established that fish have a distinctive gut micro flora, for example recent studies have shown that the gastrointestinal tract of marine fish of various species contain bacterial strains that belong to two specific *Vibrio* groups (44)

When grown on solid media, *Vibrio* strains isolated from the endogenous gut micro flora of healthy fish of various species appear to influence the growth of each other as well as to produce substances inhibitory to *Vibrio anguillarum* & *Vibrio salmonicida*(44)

Fish occupies the major source of protein in our diet and earn foreign exchange through export, these fish are often infected with or may harbor various bacteria which may cause health hazards to the fisherman, fish handlers and even to the consumers.

In addition, due to unhygienic treatment & bad sanitation both local and exportable fish were often contaminated with a variety of pathogenic bacteria which may also cause threat to human health(45)

Various studies from different part of the world have demonstrated that many enteric organisms like *Aeromonas*, *Plesiomonas*, *Vibrio*, *Salmonella edwardsella*, *Escherichia* are associated with fish and initiate various fish-borne disease in man(45) Human infections caused by pathogen transmitted from fish or the aquatic environment are quite common depending on the season, patients contact with fish and related environment, dietary habits and the immune system status of the exposed individual, there are often bacterial species facultatively pathogenic for both fish and man and may be isolated from fish without apparent symptoms of disease(46)

The infection source may be fish kept either for food or as a hobby(47)

Through anamnesis and microbiological examination are the prerequisites for correct diagnosis, however, quantification of the occurrence of these disease is difficult because many cases, typically gastrointestinal illness, go unreported the symptoms usually do not last long and are self-limiting in healthy people.

It can be extremely difficult to detect certain *in vitro* slow growing causative agent of disease such as those of mycobacterial infections or infections caused by anaerobic pathogens mycobacterial infections are quite often misdiagnosed with subsequent inappropriate therapy.(48). the presence of *Escherichia coli* is currently used as an indicator of recent fecal contamination. *Aeromonas* has received particular attention because of its association with human disease(45).(49) isolated *Aeromonas* spp. From healthy fish. (45) isolated *Aeromonas* spp.(69.2%) *Vibrio* spp.(42.3%) *Plesiomonas shigelloides*(35.9%) and *Escherichia coli* (16.7%) from fresh water fish of different categories collected from market of Dhaka city of Bangladesh. The presence of fecal coli form bacteria in the intestinal tracts of fish that inhabit polluted water is well documented. *Aeromonas* spp. Isolates from river water in Volga delta from fish, raw meat and from patients with diarrhea (50) (14) isolated *aeromonas hydrophila* from fish sold in Gaborone, Botswana supermarkets.

Salmonella Arizona, *Salmonella Paratyphi*, *Vibrio cholera*, *Proteus mirabilis*, *Proteus vulgaris*. The high bacterial counts in the supermarkets suggested poor handling during the gutting and scaling process by vendors. According to (51) poor hygiene and unsanitary handling of food are the causes of contamination of food. The environments attracted flies and insects which might also have contributed to the higher bacterial counts. Several factors like time taken during the processing, time taken to transport and trade the fish might contributed to the higher bacterial counts. The very presence of coli form above the acceptable limit in fish marketed on the streets presents a risk to

the consumers this because coli forms grow rapidly at ambient temperatures and spoil the fish in a short period of time.(14)

The growth of Salmonella spp. As well the cross contamination from viscera to flesh during processing.

Unhygienic processing conditions and absence of clean water during degutting might have contributed to the contamination followed by growth of the bacteria.

The presence of fecal coli form bacteria in the intestinal tracts of fish that inhabit polluted waters is well documented(52,39) fecal coli forms in fish are influenced by fish feeding habits and fish can harbor fecal coli forms up to 14 days after being exposed to contaminated water.(53)

(53)isolated E.coli from fish fecal materials 60% were from various animal hosts and the remaining 40% likely originated from sediments.

(54)isolated 18 enterotoxigenic strains of E.coli from (3) of 24 samples of fresh fish originating from Brazilian markets.

(55)isolated E.coli, Salmonella, Proteus spp. And Pseudomonas spp. Isolated from 62% of fish, the isolation of these groups of organisms indicated fecal and environmental pollution. (56)who isolated similar organisms from fish and fish products. The isolation of Pseudomonas spp. From fish is highly importance because this bacterium plays a considerable role as potential pathogens bacteria for human and as indicator of food quality as spoilage organism.(57) The high prevalence of staphylococcus in fish samples indicated the un hygienic handling of the fish since these species are found on human skin(14) Vibrio spp. Isolated from fish in marine fish(58).

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