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A Study on Bacteria Related to Fresh-Water Cultivation Tilapia Fish

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ABSTRACT

Nile fish genus (Oreochromis niloticus) production is graded on the highest of cultivation fish in Egypt. This study was aimed to screen microorganism related to cultivation fresh-water fish genus to see the microbiological safety of those wide distributed fish in Egypt. The mean viable microorganism count from fish fillets with the skin samples unconcealed five.6 ± 0.8 logs CFU/g. a complete of 11 (11) microorganism species were isolated and known including: E. coli, E. coli O157: H7, enterics enterica, Morganella morganii, Proteus genus Mirabilis, Proteus vulgaris, Enterobacter cloacae, Enterobacter cancerogenus, Hafnia alvei, Aeromonas hydrophila, Photobacterium damaselae. The frequency of incidences of the isolated microorganism indicated that Enterobacter cloacae had the very best frequency of occurrence (12%), whereas one isolate (2%) of enterics enterica, E coli O157:H7, and Aeromonas hydrophila were detected. These microorganism species are doubtless unhealthful to humans. Therefore, sanitary handling strategies and correct process are required before consumption of this fish.

Key words: Microbiological safety, Nile River fish genus, unhealthful microorganism

1. Intruduction

Aquaculture is considered one amongst the quickest growing aspects of agricultural trade over the globe. With increasing demand for fish and alternative food merchandise, cultivation has the potential of changing into a very important various offer of those merchandise (Lucas, 2003). fish genus fish is wide mature farmed fish and is considered the second most vital cluster when carps. In 2004, fish genus was graded the eighth most well-liked among all food within the USA world. Production of fish genus (all species) was calculable one.5 million tons in two003 and enhanced to 2.5 million tons by 2010. Most of this increased production is predicted to be attributed to Nile River fish genus. Egypt is that the world's second largest producer of farmed fish genus

when China [1]. Egypt has the most important cultivation trade in continent that has concerning 75.46 you look after the country's fish production [2]. Nile River fish genus production is graded on the highest of cultivation fish in Egypt, its production enhanced from 557,049 tons in 2010 to 768,752 in 2012 [3].

Major pathogens that are poignant the cultivation embrace microorganism, fungi, viruses, and parasites [4-6]. microorganism diseases became major concern to cultivation, particularly with heat water temperature [7]. totally different microorganism species were reported unhealthful to aquatic fish genus, as well as Aeromonas hydrophila, Edwardsiella tarda, Flavobacterium columnare, bacteria spp., Yersinia ruckeri, staphylococci epidermidis, eubacterium vulnificus, and eubacteria agalactiae [8-16].

According to information from the Centers for malady management and hindrance (CDC) [17] fish was joined to pure gold of foodborne malady outbreaks and 6 June 1944 of all sickness, or foodborne malady. Level of microorganism pathogens in fish genus fish was associated with surroundings and handling before their arrival to grocery store and restaurants. microorganism related to fish genus fish can be transmitted to person in grips and end in foodborne malady. as an example, handling fish genus was reported related to eubacterium vulnificus irruption in Seattle grocery [18]. alternative foodborne unhealthful microorganism as well as enterics enterica, enteropathogenic escherichia, L. monocytogenes, Yersinia enterocolitica, and enterobacteria pneumoniae were known from recent Nile River fish genus in Kenyan H_2O fish chains [19]. Shigatoxigenic and enteropathogenic escherichia were isolated from farmed fish genus fish (Oreochromis niloticus) in northeast region of city state [20]. watching microorganism related to cultivation fish genus fish of nice importance to public health as a result of the assist perceive microorganism medical specialty in fish and demonstrate however they'll transmit food borne malady associated with fish to humans. The aim of this study was to screen microorganism related to cultivation fish genus to see the microbiological food safety of those wide distributed fish in Egypt.

2. Materials And Strategies

A total of fifty healthy H2O fish genus fish (Oreochromis niloticus) were at random chosen from a poster fish farms placed in metropolis, Egypt. All fish samples throughout assortment were placed in sterile polypropene baggage, placed in cinnamene box containing crushed ice and therefore the temperatures were between four °C and half-dozen °C throughout transportation. Ice was ready in laboratory exploitation sterile distilled deionized water. Samples were transported to the laboratory and examined forthwith.

2.1 Bacteriological Analysis

Fish samples were processed in complete sterile condition. Fish were filleted wherever skin half were unbroken with the flesh exploitation sterile knifes and extractor and placed on sterile receptacle. Samples (25 g) were homogenized for two min. in a very sterile bag containing 225 cubic centimeter of buffered organic compound water (0.1%) (Lab M, UK) employing a garment (Seward garment four hundred circulator, UK). when incubation for 18-24h at 35°C, one cubic centimeter was transferred for more analysis from this nonselective pre-enrichment.

2.2 Aerobic Plate Count

Serial dilution 1:10 folds were then performed for total aerobic microorganism count. Dilutions were spread-plated onto plate count agar (lab M, UK) and incubated at 35 nightlong. Readings obtained inside 25 to 250 colonies on a plate were accustomed calculate microorganism population numbers and reported as logs of colony forming units (CFU/g). Experiments were continual and results were diagrammatic as suggests that ± normal deviations.

2.3 Isolation and Identification of Microorganism

E. coli O157: H7 were known by transfer preindictment culture (1 ml) to escherichia selective broth supplemented with antibiotic (EC+n) [21]. A loopful of culture was patterned onto a chromogenic selective agar (Lab M, Lancashire, UK), and sorbitol MacConkey agar (SMAC, Lab M, Lancashire, UK). Violet-colored colonies are going to be chosen on the chromogenic selective agar. Non-sorbitol chemical process E. coli O157:H7 turn out pale, colorless colonies are going to be chosen from SMAC [22]. Suspect colonies are known biochemically with indole kovac's chemical agent (Merck, Germany), Simmon change state agar (Lab M, UK), Methyle red (MR, Lab M, UK), and Voges–Proskauer (VP, Lab M, UK) tests. Species were identification consistent with authority bacteriologic analytical manual (US-FDA, 2007). more identification was done exploitation API 20E diagnostic strips (Biomérieu, Marcy, France).

Pre-enrichment culture (1 ml) were transferred into tetrathionate broth (TT, Lab M, UK) and incubated at 37°C for twenty-four h for enteric bacteria spp. isolation and identification. Culture were streaky onto sugar essential amino acid deoxycholate agar (XLD, Lab M, UK) and

incubated at 37°C for twenty-four h. Red colonies with black center from XLD media were elect and streaky on trypticase soy agar slants (TSA, Lab M, UK). once incubation bacterium was subjected to organic chemistry tests: indole, citrate, triple sugar iron (TSI, Lab M, UK), enzyme (Lab M, UK), and identification with API 20E diagnostic strips as delineate in printed reports (Food and Drug Administration).

Colonies of various characteristics of form, size, and color were elect indiscriminately from plate count agar and incubated on further Trypticase soy agar (TSA, Lab M, UK) slants. All the refined isolates were discovered for Gram staining and cell morphology. The isolates were then known biochemically with indole kovac's chemical agent, Simmon change state agar, MR, VP tests, triple sugar iron (TSI, Lab M, UK), and H2S production for identification to genus or species level in parallel, the business API 20E strips were additionally used.

2.4 Identification by PCR and 16S rRNA Sequencing

Randomly elect samples were used for this method either for confirmation of API results or for identification of unknown samples. The technique was performed in line with Azwai1 et al. deoxyribonucleic acid extraction was done exploitation microorganism deoxyribonucleic acid preparation kit (Jena life science, Thuringia, Germany). Partial 16S rDNA was amplified exploitation the universal oligonucleotides primers forward 5'-GAGTTTGATCCTGGCTTAG-3' and reverse 5'-GGTTACCTTGTTACGACTT-3'. Briefly, two µl deoxyribonucleic acid templates (20 ng/ µl) was else to twelve.5 µl Master combine (Qiagen, Hilden, Germany) and ten.5 µl deionized binary compound for a complete volume of 25 µl. The mixture was then amplified during a deoxyribonucleic acid Thermal Cycler (Techne Progene, Marshall Scientific, Hampton, NH) exploitation the subsequent program: one denaturation step at 94°C for five min; thirty seven cycles (30s at 94°C, 30s at 51°C, and 30s at 72°C); and a final extension for five min at 72°C. Gel analysis of the PCR merchandise were performed by gel activity exploitation one.5% Agarose gel with 1X Tris-acetated (TAE) buffer.

2.5 DNA Sequencing

QIA-quick Kit (Qiagen, Hilden, Germany), was used for purification of the PCR merchandise. Second PCR was performed exploitation BigDye killer v3.1 Cycle Sequencing Kit. every reaction (20 µL) contained a killer prepared reaction combine (8 µL), Primer (3.2 pmol), deoxyribonucleic acid example amounts in line with the PCR product size, and deionized water. Thermal profile for Cycle Sequencing PCR was one min at 96°C; 25°C cycles as follows: ten s at 96°C, five s at 50°C, and four min at 60°C. once an extra step of purification with CENTRI-SEP Columns (Princeton Separations, Freehold, NJ), deoxyribonucleic acid sequencing was allotted by 3500 Genetic analyzers (Applied Biosystems, Massachusetts, USA). The obtained agreement sequences were subjected to BLAST search through the Mega program (7.0.20).

3. Results And Discussion

Quantitative estimation of aerobic bacterium in genus Tilapia fish samples were calculable five.6 ± 0.8 logs CFU/g. the full aerobic count were at intervals the suitable limits compared to the Egyptian Organization for Standardization, EOS Still, the microorganism load all told samples was high and one amongst the explanations is also that the hot temperature as fish were collected in summer wherever temperature vary from 30-35°C. This temperature was mirrored on the water setting to be on the point of optimum for several mesophilic bacterium and increase the microorganism load in fish. In agreement with this results, genus Tilapia (Oreochromis niloticus) were rumored related to high total microorganism count isolated from fish surface returning from the northern region of Costa Rica, and up to twenty seven.3 x 108 CFU/g sold-out at Sokoto Central Market in Sokoto, Nigeria rumored even higher microorganism load (5.5 x 109 CFU/g) of genus Tilapia fish than this study. The high microorganism calculates the skin can be thanks to contamination by original aquatic species moreover as trade goods contamination throughout handling.

E. coli isolates (4/50, 8%) has been recovered from this study, and just one isolate (1/50, 2%) was known as E. coli O157:H7 (Figure 1). E. coli O157:H7 was isolated and known as gram negative sorbitol nonchemical process colonies on SMAC plates. Isolates were confirmed by organic chemistry tests listed in Table 1, and by exploitation medical science blood test against O157, and H7 specific antisera. For more confirmation, E. coli isolate was known by PCR and 16S rDNA sequence sequencing. The ester sequence of Escherichia coli O157: H7 Ras4 has been submitted to the GenBank with accession range KY120324 and depicted during a phylogenic tree in Figure 2. E. coli O157:H7 isn't unremarkably rumored related to fish and food. E. coli were isolated in 414/484 finfish samples in Republic of India however typical E. coli O157 was absent, but MUG and sorbitol-negative strains were rumored. Thampuran et al finished that, this result may counsel the existence of the strain. On the opposite hand, Surendraraj et al. recovered E. coli O157:H7 from shellfish in

Table 1: Biochemical test results of different isolated bacteria using API 20E diagnostic strips.

		Morganella morganii	*Proteus mirabilis*	*Proteus vulgaris*	*E. coli*	*Salmonella enterica*	*Enterobacter cloacae*	*Enterobacter cancerogenus*	*Hafnia alvei*	*Aeromonas hydrophila*	*Photobacterium damaselae*
ONPG		-	-	-	+	+	+	+	+	+	-
Arginine dihydrolase		-	-	-	+	-	+	-	-	+	+
Lysine decarboxylase		-	-	-	+	-	-	-	+	+	-
Ornithine decarboxylase		+	+	-	-	+	+	-	+	-	-
Citrate		-	+	-	-	+	+	+	+	-	-
H 2S		-	+	+	-	+	-	-	-	-	-
Urease		+	+	+	-	+	-	-	-	-	+
TDA		+	+	+	-	-	-	-	-	-	-
Indole		+	-	+	+	+	-	-	-	-	-
Voges-Proskauer		+	-	-	-	-	+	+	+	+	-
Gelatinase		-	+	-	-	-	-	-	-	+	-
Acid from:	glucose	+	+	+	+	+	+	+	+	+	+
	Mannitol	+	-	-	+	+	+	+	+	+	-
	Inositol	-	-	-	-	-	-	-	-	-	-
	Sorbitol	-	-	-	+	+	+	-	-	-	-
	Rhamnose	-	-	+	+	+	+	+	+	-	-
	sucrose	-	-	+	+	+	+	-	-	+	-
	Melibiose	-	-	-	+	+	+	-	-	-	-
	Amylose	-	-	-	-	+	+	+	-	-	-
	arabinose	-	-	-	+	+	+	+	+	-	-

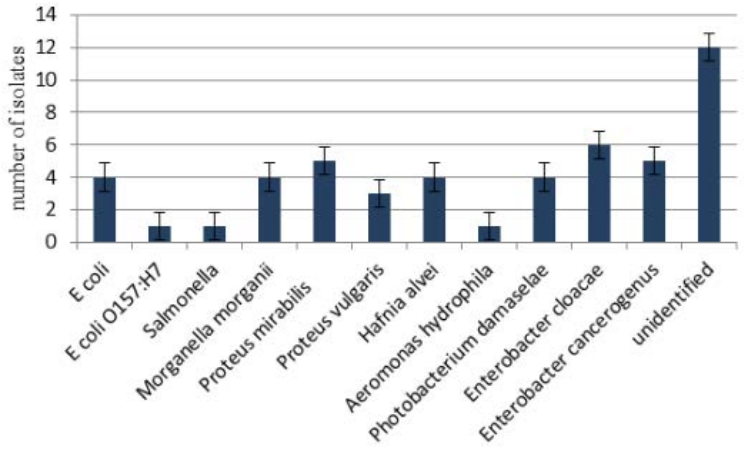
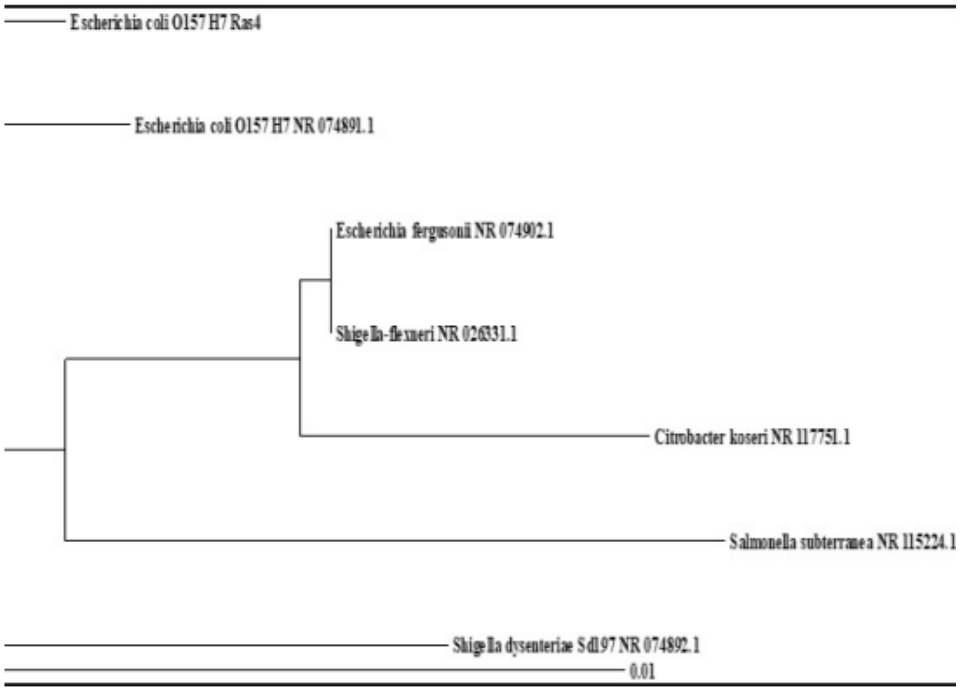


Figure 1: Number of occurrence of bacteria isolated from fresh water aquaculture tilapia (Oreochromis niloticus) fish.

food markets in Republic of India. Seven commonplace E. coli O157:H7 were known and one shrimp sample was positive for three virulence markers. Another study conducted on Nile genus Tilapia (Oreochromis niloticus) fish skin, canal, and muscles from pay-to-fish ponds placed in at the Córrego RICO watershed in metropolis, Brazil isolates. 8 from 96 totals E. coli isolates (6.95%) from the fish canal contained O157 sequence, but E. coli O157 wasn't rumored on fish skin. Shiga toxin-producing Escherichia coli (STEC) O157 were rumored recovered from processed salmon roe that had been a suspected food item in infrequent infections occurred in Japan, 1998.

Wang and Doyle reported that E. coli O157 will survive in water for many weeks. Therefore, unclean contamination of water sources or cultivation environments by E. coli O157 will act as a vehicle of transmission of diarrheagenic enteric infections. Water provides were reported contaminated by E. coli O157 in Brazil and European country. could|this might|this could} suggests that fish contamination may originate from bovine body waste, most likely from the encircling to cultivation water. E. coli bacterium that may cause human diseases don't cause losses in cultivation production. Therefore, fish farmers do not feel the requirement to use applicable health management measures

Figure 2: Phylogenic tree represented sequenced Escherichia coli O157: H7 Ras4 (GenBank accession number KY120324).



to make sure product quality. However, infected fish used as a food supply will function means that of transmission of those agents to humans. Therefore, sensible sanitary handling measures and correct process are required before consumption of fish product.

Salmonella spp. (1/50, 2%) was isolated and known during this study (Figure 1). Isolates were gram negative black focused colonies on XLD media. elect colonies were tested by indole, citrate, MR, VP, urease, and TSI organic chemistry tests and known with API 20E as enterics enterica as listed. enterics causes foodborne malady related to dehydration, reactive inflammatory disease, septicemia, and might cause death. Therefore, Food safety standards have demanded the absence of enterics in chilled cannon fodder. enterics may be introduced to the aquatic system through some ways like poor sanitation, inappropriate disposal of human and animal wastes. The presence of enterics enterica within the gift study recommended the existence of poor sanitary measures and higher management and observation is needed.

Ellermeier and Slauch discovered that cold-blooded animals like Tilapia by themselves are potential hosts for enterics species. enterics are reported related to Tilapia fish in many studies. completely different enterics serovars (S. Corvallis, S. Bovis-mobificans, S. Agona, S. Mikawashima, and S. Typhimurium) were isolated from Tilapia (14/32, 43.8%) obtained from wet markets in Asian country. enterics spp. were found in fish secretion (20.0%) of a complete of twenty Tilapia. enterics spp. were reported in raw retail frozen foreign fresh-water Tilapia fish to japanese province of Kingdom of Saudi Arabia sixty fourth (16/25) from Asian nation, and twenty eighth (14/50) from Republic of India. Similar results to the current study were discovered with recent Tilapia fish (Oreochromis niloticus) in Sokoto, Nigeria. enterics spp. Showed the smallest amount frequency of incidence (1/31, 3.22%).

Several bacteria isolated during this study have the potential of amine formation. These bacterium embody Morganella morganii (4/50, 8%), Proteus genus Mirabilis (5/50, 10%), Proteus vulgaris (3/50, 6%), Hafnia alvei (4/50, 8%), Aeromonas hydrophila (1/50, 2%), Photobacterium damaselae (4/50, 8%), Enterobacter cloacae (6/50, 12%), and Enterobacter cancerogenus (5/50, 10%) (Figure1). All isolates were confirmed by organic chemistry tests listed, and for additional confirmation, Morganella morganii isolate was known by PCR and 16S rDNA sequence sequencing. The ester sequence of Morganella morganii Ras1 has been submitted to the GenBank with accession variety (KY120325) and described in a very phylogenic tree in Figure 3. Same confirmation was finished Proteus genus Mirabilis isolate, because it was known by PCR and 16S rDNA sequence sequencing. The ester sequence of Proteus genus Mirabilis Ras2 has been submitted to the GenBank with accession variety (KY120326) and described in a very phylogenic tree.

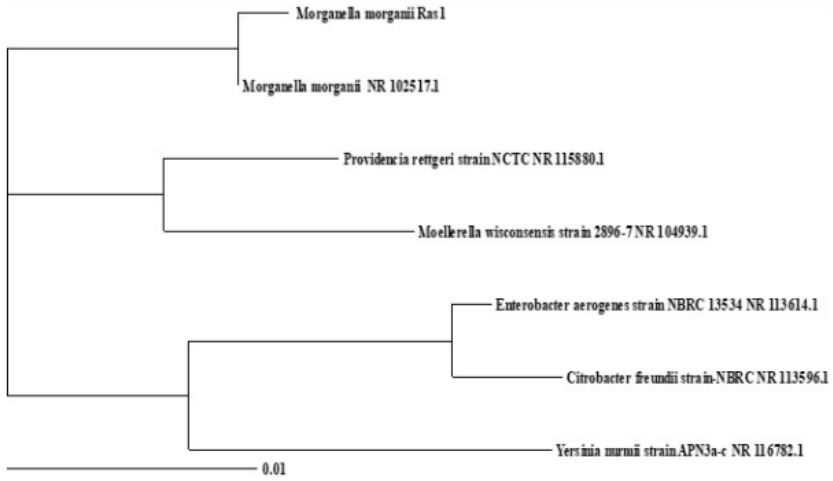


Figure 3: Phylogenic tree represented sequenced Morganella morganii Ras1 (GenBank accession number KY120325).

Histamine production is related to percoidean poisoning, and its toxicity is increased by the presence of different biogenic amines in foods. amine is created by chemical action of essential amino acid, that is found at high levels in muscles of fish happiness to the Scombroidae family. It usually results from proliferation of amine manufacturing bacterium, that possess essential amino acid enzyme. bacteria family has been reported to be the foremost vital histamine-producing bacterium in fish. M. morganii, Proteus app., and Hafnia alvei are thought of proliferating amine forming bacterium and therefore the amount of amine made is varied among species reported Photobacterium spp. and Aeromonas spp. as amine producers. Tilapia failed to belong to the Scombroidae family, however all food made in supermolecule are prone for amine and different biogenic amines formation once fascinating conditions are gift for the organism. amine was calculable in fifty two Tilapia fish in Bahir Dar city, Ethiopia. Mean level of amine detected was between three.8-290 mg/100 g, that exceed the accepted limit of amine established by EU regulation and will cause amine toxicity.

Bacteria isolated during this study related to completely different complications which may have an effect on the general public health. P. genus Mirabilis becomes AN timeserving microorganism wherever it causes tract infections and different forms of healthcare facility infections. Hafnia alvei reported related to persistent septicaemia. consistent with Kirov. Aeromonas spp. are pathogens which may cause bacteraemia, meningitis, respiratory organ and wound infections. it would cause "summer-diarrhoea", that may be a worldwide drawback in kids beneath 5 years previous, the older, and travellers. Photobacterium damaselae was related to infection when digestion of raw food, and tract infection when exposure to contaminated water. it had been diagnosed with septicaemia and viscus disfunction in a very cirrhotic patient when bodily process of food. Photobacterium maid isolation from civilized fish with high amount has created the bacteria as concern in cultivation business. Abdel-Aziz et al known Photobacterium maid throughout mass mortalities of civilized seabream and European seabass in Egypt. Enterobacter cloacae are gram negative bacterium, will cause wound, metabolism and tract infections. it's thought of major human microorganism liable for giant outbreaks of healthcare facility malady. Enterobacter cancerogenus was related to septicaemia and wound infection particularly with persons exposed to the organism throughout traumatic events.

4. Conclusion

This analysis has indicated that the microorganism species related to recent cultivation Tilapia fish and has shown that they're probably infective to humans. Therefore, adequate measures ought to be taken in handling and process this wide distributed fish before consumption.

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