BACTERIOLOGICAL ASPECTS OF FRESH FRESHWATER AND FROZEN MARINE FISHES AT MANSOURA CITY

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ABSTRACT

A total of 60 sensuously sound fishes consisted of 30 fresh freshwater fishes (10 each of iced Tilapia nilotica, iced Mugil cephalus and freshly killed Clarias lazera) besides 30 frozen marine ones (10 each of Mackerel, Sardine and Herring), were randomly purchased from different markets in Mansoura city. Each fish was represented by aseptically excised back 60 g-muscle. All excised samples were tested bacteriologically.

Bacteriological analyses showed the presence of aerobic mesophiles in all (100%) muscle samples by mean levels of $2.8x10^4$, $2.6x10^4$ and $4.6x10^4$ organisms/g of fresh freshwater fishes alongside $5.3x10^3$, $6.4x10^3$ and $6x10^4$ organisms/g of frozen marine fishes; whereas, psychrotrophic bacterial contamination were recognized in 8 (80%) - 10 (100%) of the same samples by mean of $3x10^4$. $2.3x10^4$ and $1.2x10^4$ as well as $3.6x10^3$, $4.7x10^3$ and $4.7x10^2$ organisms/g. Similarly, Enterobacteriaceae contamination were recovered from the muscles of 6 (60%)-8 (80%) and 9 (90%)-10 (100%) of both fishes' samples by mean levels of $2.6x10^2$ $2.4x10^2$, $7.1x10^2$ and $4.3x10^2$, 8.3x10, $1.1x10^2$ organisms/g, consecutively; while, Staphylococcus aureus organisms could not detected in all examined samples. Bacteriological analyses could also detect the contamination with Aeromonas organisms in 6 (60%) - 8 (80%) of fresh freshwater in addition to 7 (70%) – 10 (100%) of frozen marine fishes by mean of $2x10^2$, $3.6x10^2$, $3.5x10^2$ and $3.9x10^2$, $3.1x10^2$, $4.2x10^2$ organisms/g, successively. Salmonellae were exclusively found only in one (10%) every of samples taken from iced Tilapia nilotica and Mugil cephalus alongside freshly killed Clarias lazera. The all three strains, obtained from the identical number of aforementioned samples, were serologically identified as Salmonella Kottbus. An exclusive investigation of vibrios organisms revealed their occurrence only in one (10%) frozen Mackerel plus 2 (20%) frozen Sardine samples, identified as one V. cholerae plus 2 V. parahaemolyticus strains.

Microbiological risk assessment of all surveyed fishes, through comparing different bacterial populations contained in their tissues with the relevant limits, showed that 3 (30%) out of the overall 30 fresh freshwater fish muscle samples and 1 (10%) out of 10 frozen Sardine muscle samples were harboured Vibrio cholerae organisms, thus exceeded the recommended limit (free for both organisms), whereas none of the

tested samples exceeded the recommended limit for aerobic mesophiles, Enterobacteriaceae or Staphylococcus aureus organisms.

The types and numbers of bacterial strains-obtained from the surveyed flesh samples of fresh freshwater and frozen marine fishes- after being picked up randomly from the separate colonies each of psychrotrophs grown onto plate count agar, presumptive Aeromonas spp on starch-ampicillin agar, presumptive Salmonella spp on XLD agar and Vibrios spp on TCBS agar, a total of 73 strains distributed as 2 strains of Acinetobacter calcoaceticus; 2 and 2 Aeromonas hydrophila and Aeromonas salmoncida; 2 Alcaligenes faecalis; 4 and 3 Bacillus cereus and Bacillus circulans; 1 Branhaemlla catarrhalis; 4 and 3 Chromobacterium lividum and Chromobacterium violaceum; 4 Citrobacter freundii; 2 Corynebacterium ulcerans; 2 and 5 Enterobacter agglomerans and Enterobacter cloacae; 3 Escherichia coli; 3 and 2 Micrococcus luteus and Micrococcus varians; 2 Niessera flavescens; 1 Proteus vulgaris; 5, 3 and 4 Pseudomonas aeruginosa, Pseudomonas alcaligenes and Pseudomonas fluorescens; 3 Salmonella Kottbus; 1 Serratia liquefaciens; 4 and 3 Staphylococcus aureus and Staphylococcus epidermidis besides 1 and 2 strains of Vibrio cholerae and Vibrio parahaemolyticus, successively.

Typing of Enterobacteriaceae organisms- picked up randomly from the separate colonies grown onto plates of violet red bile glucose agar and obtained from flesh samples of all tested fresh freshwater and frozen marine fishes- showed a sum of 36 Enterobacteriaceae strains identified as 5, 1 and 1 strains of Citrobacter freundii, Citrobacter diversus and Citrobacter morganii; 4, 2 and 8 Enterobacter agglomerans, Enterobacter cloacae and Enterobacter hafniae; 4 Escherichia coli; 2, 1 and 2 Proteus mirabilis, Proteus rettgeri and Proteus vulgaris; 1 Provedencia alcalifaciens plus 3 and 2 strains of Serratia liquefaciens and Serratia rubidaea, consecutively.

INTRODUCTION

Fish are nutritious food that constitute a desirable components of healthy diet and considered an excellent source of high quality protein containing a good balance of essential amino acids and having a high biological value and containing lipids with high levels of unsaturated fatty acids which contributing to the enhancement of human health by reducing the risk of cardiovascular disease. Likewise, fish are characteristically tender, easily digest and good source of most B-complex vitamins and also contribute significant levels of minerals including cobalt, iron, copper, zinc, sodium, potassium, magnesium, phosphorous, iodine and fluorine. Familiarity, taste, low-cost and convenience are some of the appealing factors that make fish a substitute for the red meet as a source of animal protein, providing an opportunity for consumers to meet their daily nutritional requirements **(NRC, 1998)**.

Although, the flesh of freshly-caught fishes is sterile and bacteria are only found, in variable numbers, on their skins and gills as well

as in their intestines **(Hayes, 1992)**, most fishes are exposed to animal and/or human wastes from the local environments and can easily be contaminated by a wide variety of pathogenic organisms, thus fishes and other creatures that inhabit waters close to the shores are particularly susceptible to bacterial contamination contained in raw sewage, such as Salmonella and Vibrio organisms **(Satin, 2008)**. The contamination by these pathogens originates mainly from: (1) pathogens are naturally inhabitant in seafoods or in the marine and fresh water environment as (Vibrio and Aeromonas spp), (2) fecal pollution (Salmonella spp, Shigella spp and fecal coliforms) and (3) processing, handling and preparation (V. parahaemolyticus, V. cholerae, Salmonella spp, Shigella spp and Staphylococcus aureus). Many of these pathogens pose a slight risk to normal human populations, but all pose serious risk to specific population groups such as persons with defects in their immune systems **(Archer and Young, 1988)**.

Therefore, the overall objectives of this work were intended to assess the microbiological risk in raw flesh of some common freshwater fishes (iced Tilapia nilotica and freshly killed Clarias lazera plus Mugil cephalus), besides that of some frozen marine fishes (Mackerel, Sardine and Herring) widely consumed in Egypt through fulfilling the following points: (1) Estimation of the total bacterial counts of aerobic mesophiles (APC), psychrotrophs, Enterobacteriaceae, Staphylococcus aureus, Aeromonas spp and halophiles per each gram of the examined fish muscles, (2) Detection and serotyping of the Salmonella organisms particularly those usually implicated in food-poisoning outbreaks among consumers, (3) Detection and identification of Aeromonas spp particularly pathogenic and food-poisoning types and (4) Detection and identification of Vibrio spp particularly V. parahaemolyticus and V. cholerae.

MATERIALS AND METHODS

[A] COLLECTION OF SAMPLES :

A total of 60 sensuously sound fishes consisted of 30 fresh freshwater fishes (10 each of iced Tilapia nilotica, iced Mugil cephalus and freshly killed Clarias lazera) besides 30 frozen marine ones (10 each of Mackerel, Sardine and Herring), were randomly purchased from different markets in Mansoura city. Each sampled fish was aseptically and individually packed into a polyethylene bag then marked and transferred -in icebox with a minimum of delay- to the laboratory of Food Hygiene and Control Department, Faculty of Veterinary Medicine, Mansoura University, wherein the bacteriological analyses were done.

[B] PREPARATION OF SAMPLES (ICMSF, 1986):

A sum of 60 g-back was muscle were aseptically excised from every sampled fresh freshwater fish then divided into 3 portions, meanwhile only 35 g-back muscle was similarly taken from frozen marine fish and divided into 2 portions. The first 10 g-muscle portion of each of all fishes was homogenized with 90 ml of 0.1% sterile peptone water (Oxoid CM0009) in a laboratory blender (Type: Moulinex-made in France)/one min for obtaining an original dilution of 1:10. One ml from each of the original dilutions was transferred to sterile test tubes containing 9 ml of the same

diluent to be diluted in a sequential manner preparing a tenfold serial dilutions up to 106 for bacterial enumeration of aerobic mesophiles, psychrotrophs, Enterobacteriaceae, Staphylococcus aureus and Aeromonas in surveyed muscles. The second 25 g-muscle portion, excised from freshwater fishes was investigated for presence of salmonellae by pre-enriching it in 225 ml of sterile buffered peptone water (Oxide CM0509B) for 24 h at 37°C after being blended for one min, whilst identical 25 g-muscle portion taken from frozen marine fishes was directed for detecting both vibrios and Aeromonas organisms through enriching it in 225 ml of sterile alkaline peptone water (APW), pH 8.6 (Oxoid CM1028B) for 8 & 24 h at 37°C after blending them for one min. Finally, the third 25 g-muscle portion, only derived from freshwater fishes, was consigned to recognize Aeromonas organisms via enriching it in 225 ml of sterile alkaline peptone water, pH 8.6 (Oxoid CM1028B) for 24 h at 37°C.

[C] BACTERIOLOGICAL ANALYSES:

(1) Aerobic plate count (ICMSF, 1986):

A tenth ml from each prepared serial dilution was transferred and evenly spread over a dry surface of duplicated, previously prepared sterile plate count agar medium (Oxoid CM0325B). The surface of inoculated plates was allowed to dry for 15 min before being placed inverted with control plates in the incubator adjusted at 30°C for 2 days. The bacterial colonies in the countable plates (having 30-300 colonies) were enumerated and the aerobic plate count per each g of the examined samples was calculated and recorded.

(2) Total psychrotrophic count (Cousin et al., 1992):

The same procedures were taken place as for counting APCs with the exception of incubation temperature / time were 5°C/7 days. Separate colonies of different morphological characters, previously grown on plate count agar plates used for enumeration of psychrotrophs, were picked and inoculated into tryptone soya broth then incubated at 37°C for 24 h, and purified onto nutrient agar slants for identifying them by using different biochemical tests, according to **Holt et al. (1994)**.

(3) Enterobacteriaceae count (ISO, 1993):

Duplicated sets of sterile Petri dishes were inoculated with 1-ml amounts of the chosen range of prepared dilutions. quantity of about 15 ml of violet red bile glucose agar (Oxoid CM485B), melted and cooled to 45°C, was added to each inoculated Petri dish, then mixed well and allowed to set. Another 5 ml of the same agar/temperature was finally overlain every plate, which left to be solidified, then incubated "inverted" at 30°C for 24 h. Typical colonies of Enterobacteriaceae (red surrounded by precipitation of bile salts in the medium and having 0.5 mm or more in diameter) were enumerated in the countable plates (having 25-250 colonies) and the Enterobacteriaceae count per g of the examined sample was calculated and recorded. Separate colonies were picked and inoculated into tryptone soya broth and incubated at 37°C for 24 h, then purified onto nutrient agar slants for identification according to **MacFaddin (1976)**.

(4) Staphylococcus aureus count (AOAC, 1984):

From the previously prepared dilutions, 0.2 ml from the selected dilutions was transferred and evenly spread onto dried surfaces of duplicate plates of Baird-Parker (B-P) selective agar (Oxoid CM275) with egg-yolk tellurite emulsion. Inoculated plates were allowed to dry then incubated at 37°C for 48 h. Colonies exhibiting typical morphology, grey-black to jet black, circular, smooth, convex, 2-3 mm in diameter with a narrow white entire margin surrounded by a zone of clearing extended 2-5 mm in the opaque medium, the Staphylococcus aureus count per g of the examined sample was calculated and recorded.

(5) Enumeration and identification of Aeromonas species (Palumbo et al., 2001):

1- Enumeration of Aeromonas species:

From the previously prepared dilutions, a tenth ml from the selected dilutions was transferred and evenly spread onto dried surfaces of duplicate plates of starch-ampicillin agar. Inoculated plates with control one were allowed to dry then incubated at 28°C for 24 h. Colonies exhibiting typical morphology (yellow to honey colored, 3 to 5 mm in diameter and having a clear zone surrounding the colony "amylase-positive" after flooding plates with 5 ml Lugol iodine solution) were scored as presumptive Aeromonas species. Five of these colonies were picked up, purified onto nutrient agar slopes, incubated at 37°C /24 h and subjected for confirmatory biochemical identification. The total Aeromonas count per g was then calculated and recorded.

2- Identification of Aeromonas species:

Aseptically, 25 g muscle sample was thoroughly blended with 225 ml sterile alkaline peptone water (APW) having pH 8.6 (Oxoid CM1028B) for one min then incubated at 37°C for 24 h. A loopful from each of the enriched homogenate was inoculated onto the dried surface every of duplicate plates of starch-ampicillin agar which then incubated at 28°C for 24 h. Five presumptive Aeromonas colonies (yellow to honey colored, 3 to 5 mm in diameter and having a clear zone surrounding the colony "amylase-positive" after flooding plates with 5 ml Lugol iodine solution) were picked up, purified onto nutrient agar slopes, incubated at 37°C for 24 h and directed for confirmatory identification by using different biochemical tests.

(6) Isolation and identification of salmonellae (AOAC, 1995):

Aseptically, 25 g of each fish muscle was thoroughly mixed with 225 ml sterile buffered peptone water (Oxoid CM0509B) for one min then the homogenate was incubated at 37°C for 18-24 h. One ml from each pre-enrichment resuscitation culture was aseptically transferred to sterile tube containing 10 ml Rappaport-Vassiliadis medium (Oxoid CM669) then incubated at 42°C for 18–24 h. A loopful from each of the enriched tubes was inoculated onto the surface of xylose lysine desoxycholate (XLD) agar plates (Oxoid CM0469B) and then the plates were incubated at 37°C for 24h. Presumptive Salmonella colonies (pink colonies with or without black centers on XLD) were picked up and purified on nutrient agar slopes and incubated at 37°C for 24 h for identification through complete biochemical tests, then presumptive

salmonellae were confirmed serologically through determination of O (somatic) and H (flagellar) antigens) by slide agglutination technique which performed, in central laboratories of health ministry in Cairo/ Egypt.

(7) Isolation and identification of Vibrio species (FDA, 1998):

Aseptically, 25 g marine fish muscle was thoroughly mixed with 225 ml sterile alkaline peptone water (APW) having pH 8.6 (Oxoid CM1028B) for one min then incubated at 37°C for 8 and 24 h. At 8 and 24 h a loopful (without shaking the flask) from each of the enriched homogenate was inoculated on to the surface of Thiosulfate Citrate Bile Sucrose (TCBS) agar plates (Oxoid CM0333B) and then the plates were incubated at 37°C for 24 h. On TCBS plate, presumptive V. cholera appeared as yellow colonies (sucrose positive), whereas probable V. parahaemolyticus showed green colonies (sucrose negative). Typical Vibrio colonies were picked up and inoculated into tryptone soya broth with 3% NaCl, incubated at 37°C for 24 h, purified onto nutrient agar slants with 3% NaCl and subjected for confirmatory biochemical tests by using different media contained 2.5% NaCl.

The data obtained in this study were statistically analyzed according to methods described by **Snedecor (1989)**.

DISCUSSION

Inspection of data arranged in Table (1) exhibits the omnipresence of aerobic mesophilic bacteria in flesh of all surveyed fishes, as they found in 10 (100%) every of fresh freshwater fishes; iced Tilapia nilotica, iced Mugil cephalus and freshly killed Clarias lazera, as well as of frozen marine fishes; frozen Mackerel, frozen Sardine and frozen Herring, were harbored those organisms. **Saleh and El-Kewaiey (2008)** detected those contaminants by lower presence (88% & 92%) in flesh samples of both raw Tilapia nilotica and frozen Mackerel, respectively, whilst by similar occurrence (100%) in flesh samples of raw Mugil cephalus, whereas **Shaaban et al. (2001)** could not find bacterial contamination in any flesh sample of 75 freshly caught Tilapia fishes. These noticeable variations in occurrence of aerobic mesophilic bacteria can be explained by corresponding differences in pollution degree of catch waters as well as in handling conditions **(Huss et al., 1974 and Frazier, 1988)**.

Intensities of aerobic mesophilic bacteria were estimated in flesh samples of overall fishes, surveyed in this work, expressed as aerobic plate counts which found in fresh freshwater fishes as ranges of $3x10^2$-$1.3x10^5$ with a mean of $2.8x10^4 \pm 1.3x10^4$ organisms/g in iced Tilapia nilotica; $10^2x1.4x10^5$ with a mean of $2.6x10^4 \pm 1.4x10^4$ organisms/g in iced Mugil cephalus in addition to $2x10^2$-$1.5x10^5$ with a mean of $4.6x10^4 \pm 2.3x10^4$ organisms/g in freshly killed Clarias lazera, respectively. Whereas these levels in flesh samples taken from frozen marine fishes were $4x10^2$-$1.9x10^4$ with a mean of $5.3x10^3$ x 1.9 x 10^3 organisms/g in frozen Mackerel; $2x10^2$-3.2*104 with mean of $6.4x10^3 \pm 3x10^3$ organisms/g in frozen Sardine besides $1.2x10^3$-$2.4x10^5$ with a mean of $6x10^4 \pm 2.6x10^4$ organisms/g in frozen Herring, successively (Table 2).

Approximately similar levels of aerobic plate counts were estimated in flesh of Clarias lazera (mean of $7.8x10^4$ organisms/g) by **Morshdy (1978)**; of Tilapia nilotica and Mugil cephalus (mean of $2x10^5$ and 10^4 organisms/g, respectively) by **Saleh and El-Kewaley (2008)**; of atlantic Mackerel (range of $1.1x10^2$-$4.5x10^4$ organisms/g) by Kolodziejska et al. (2002) and of frozen Mackerel (mean of $4x10^3$ organisms/g) by Saleh and El-Kewaley (2008). On the contrary, higher intensities of general bacterial contamination were detected in flesh of Tilapia nilotica as ranges of $2x10^5$ -$4x10^7$, 103-$8x10^7$ and $7x10^4$–$2x10^6$ organisms/g by **Saleh (1990), Awad (1998) and Yagoub (2009),** whilst as mean levels of $4.6x10^5$ and $1.6x10^5$ organisms/g by **Abo Samra (2001) and Abd-Allah (2004)**, successively. Also, higher aerobic plate counts were evaluated by **Dorho (1998)** as a mean of $6.8x10^6$ organisms/g in flesh of Mugil cephalus fishes, as well as by **Dorho (1998) and Abo Samra (2001)** as mean levels of $1.15x10^7$ and $6.8x10^5$ organisms/g in flesh of Clarias lazera, consecutively. Flesh samples of some marine fishes might also contain aerobic mesophilic bacteria by higher counts than that obtained in this work, where Brando and **Furlantto (1984) and Rayasinghe and Rajakaruna (2005)** evaluated the ranges of such contaminants in flesh of 60 fishes of Sardine and Tuna by $7.9x10^3$-$4.6x10^6$ and $3.25x10^2$-$6.83x10^8$ organisms/g, respectively. Lower levels of aerobic plate counts in fish samples, identical to these investigated in this work, were obtained by many workers too; where **Saad et al. (1991)** found these counts by mean of $8.3x10^3$ and $9.9x10^3$ organisms/g of Tilapia nilotica and Clarias lazera fishes, respectively, as well as **Eves et al. (1995)** calculated the range of such contaminants in flesh of Oreochromis niloticus fishes by $3.6x10^3$-10^4 organisms/g. Also, **Ahmed and El-Khawas (2008)** detected the same organisms in flesh of frozen Salmon fishes, as a member of marine fishes, by mean levels ranged from $1.2x10^2$–$5.4x10^2$, lower than that found in flesh samples of frozen marine fishes examined in the present study.

Detailed inspection of the mean levels of aerobic plate counts, obtained from the overall flesh samples of surveyed fishes, in relation to those detected by aforementioned researchers in correspondent samples, could explain the noticeable variations among these findings by the literature of **Huss et al. (1974)** who emphasized that bacterial counts decreased in fishes caught from unpolluted waters under hygienic conditions of handling, whilst increased in fishes caught from polluted waters with bad hygienic standards on board-ship during initial handling, in addition to the declaration of **Niewolak and Tucholski (2000)** as muscles of fishes, reared in ponds regularly fertilized with treated sewage, showed increases in their content of aerobic plate counts, alongside the literature of **Liston (1980)** who assured that the aerobic plate counts on fish surfaces varied enormously; 10^2-10^7 organisms/cm^2 on skins and 10^3-10^9 organisms/cm^2 on gills, besides the reports described the ice-used for refrigerating fishes- as an additional source of fishes contamination with bacteria, by **Vieira et al. (1997) and Falcão et al. (2002)** who evaluated the ranges of aerobic plate counts in ice samples by $3.26x10^2$-$9.68x10^2$ and $1.3x10^3$-$6.1x10^5$ organisms/ml, respectively. Additionally, the fewer aerobic plate counts estimated

in flesh samples of frozen both Mackerel and Sardine fishes may be attributed to the destructive effect of freezing on some but not all microorganisms found in such fishes **(Frazier and Westhoff, 1978)**, whilst the relatively high aerobic plate counts recognized in flesh samples of frozen Herring fishes, approaching those calculated in flesh samples of fresh freshwater fishes, perhaps be due to temperature abuse prior to freezing **(ICMSF, 1986)**.

Concerning the flesh contamination with psychrotrophic bacteria in fresh freshwater fishes, plates of plate count agar medium-incubated at 5°C/7 days showed the presence of such organisms in 10 (100%) samples every of iced Tilapia nilotica, iced Mugil cephalus and freshly killed Clarias lazera by populations ranged from $1.1x10^3$-$8.9x10^4$ with a mean of $3x10^4 \pm 10^4$, $1.1x10^3$-$9.3x10^4$ with a mean of $2.3x10^4 \pm 10^4$ and 10^2-8.8*104 with a mean of $1.2x10^4 \pm 0.9x10^4$ organisms/g, respectively. Similarly, the same bacteriological technique could detect these organisms in frozen marine fishes samples in 10 (100%), 9 (90%) and 8 (80%) by counts ranged from 10^2-$2.1x10^4$ with a mean of $3.6x10^3 \pm 2x10^3$, 0-$3.2x10^4$ with a mean of $4.7x10^3 \pm 3.1x10^3$ and 0-$1.4x10^3$ with a mean of $4.7x10^2 \pm 1.3x10^2$ organisms/g of frozen Mackerel, frozen Sardine and frozen Herring, successively (Tables 1 & 2).

Mousa and Mahmoud (1997), Saleh and El-Kewaiey (2008) and Mansour and El-Shaboury (2009) could estimate psychrotrophic bacteria in flesh of Tilapia nilotica, Mugil cephalus, Clarias lazera and frozen Mackerel- collected from Egyptian fish markets- by mean levels of $3.36x10^4$ & $2x10^4$, 104, $6.6x10^4$ & $2.5x10^4$ and $4x10^3$ organisms/g, successively which were nearly similar to those correspondent levels, obtained in this work, moreover, the latter researchers found these contaminants by lower incidence (72% and 60%) in flesh samples of Tilapia nilotica and frozen Mackerel, respectively, whereas by almost equal prevalence (96%) in correspondent samples of Mugil cephalus. Higher levels of psychrotrophic bacteria were detected in flesh of iced Tilapia nilotica by **Mahmoud (1994) and Awad (1998)** as mean of $6.9x10^5$ and range of $3.1x10^3$-$9x10^6$ organisms/g as well as in flesh of iced Mugil cephalus and fresh Sardine by **Farag and Korashy (2009) and Brando and Furlantto (1984)** as ranges of $1.2x10^4$-$9x10^5$ and $3x10^3$-$3.5x10^6$ organisms/g, meanwhile, lower psychrotrophic bacterial populations were also evaluated as mean values of 5.6x10 and $3.2x10^2$ organisms/g of Tilapia nilotica flesh-es by **Mousa and Mahmoud (1991) and Mansour and El-Shaboury (2009)**, consecutively.

Seeing the obtained findings show the widespread of psychrotrophic bacteria in flesh of tested fishes by considerable numbers agreed with that reported by **Marriot (1997)** who attributed the presence of psychrotrophic bacteria in fishes muscle to contamination from the surrounding environment, processing, equipment, workers, containers and boxes, as well as with the findings of Mousa and **Mahmoud (1997)** who emphasized that the prolonged storage and long standing of fishes at shops and markets increased enormously the counts of psychrotrophic bacteria in their flesh, in addition to the results of **Falcão et al. (2002)** who estimated the range mean

levels of psychrotrophic bacterial counts in different types of Brazilian ice, used for refrigerating fishes, by 1.5x10-5.2x10^5 organisms/ml. Also, the presence of psychrotrophic bacteria in all analyzed flesh samples of frozen fishes by intensities probably approach those determined in fresh raw fishes, agreed with fact mentioned by Frazier and Westhoff (1978) who assured that psychrotrophic bacteria can survive freezing and are always ready to grow on thawing.

Regarding the contamination of surveyed fishes with Enterobacteriaceae organisms, the occurrence of such contaminants were represented by 8 (80%) samples of iced Tilapia nilotica with intensities (ranges & mean values) of 0-1.6x10^3 (2.6x10^2±1.5x10^2); by 9 (90%) samples of iced Mugil cephalus with populations of 0-7x10^2 (2.4x10^2±0.7x10^2) as well as by 6 (60%) identical samples of freshly killed Clarias lazera with levels of 0-4.2x10^3 (7.1x10^2±2.4x10^2) organisms/g, respectively. Approximate presence of these bacterias was also recognized in correspondent samples of frozen marine fishes with levels lower than those estimated in aforementioned flesh samples of fresh freshwater fishes, where it represented by 9 (90%) samples of frozen Mackerel with counts of 0-3.7x10^3 (4.3x10^2±1.6x10^2); by 6 (60%) similar samples of frozen Sardine by intensities of 0-2.9x10^2 (8.3x10±3.4x10) alongside 10 (100%) samples of frozen Herring with populations of 2x10-2.5x10^2 (1.1x10^2±0.2x10^2) organisms/g, successively (Tables 1 & 2).

Nearly equal levels of Enterobacteriaceae populations were detected in fish fillets as a mean of 10^3 organisms/g by **Brock et al. (1984);** in flesh of both Tilapia nilotica and Clarias lazera fishes as mean values of 7x10^2 and 8x10^2 organisms/g, respectively by **Abd El-Galil et al. (1988);** in Tilapia fillets as a mean of 2.04x10^2 organisms/g **Boari et al. (2008);** in flesh of Pagrus and Solea (marine fishes) as mean of 2x10^2 and 2x10^3 organisms/g, consecutively by **Sheriff (1998);** in flesh of frozen Mackerel as a mean of 3x10^2 organisms/g by **Saleh and El-kewaiey (2008).** However, the occurrence of the Enterobacteriaceae contaminants in flesh of tested fishes, obtained by many researchers, was fewer than that estimated in the present study, where **Saleh and El-kewaiey (2008)** found them in 64% and 88% flesh samples of Tilapia nilotica and Mugil cephalus, consecutively; while **David et al. (2009)** could recover these bacteria from only 52.5% similar samples of Tilapia nilotica, in addition to the finding of **Yagoub (2009)** who recognized such organisms exclusively in 55.3% of the overall samples of gills, intestine, skin and flesh taken from Tilapia nilotica fishes, alongside the lower prevalence of these contaminants as a percentage of 12% flesh samples of frozen Mackerel fishe obtained by **Saleh and El-kewaiey (2008).** On the contrary, higher Enterobacteriaceae counts were determined in flesh samples of Tilapia nilotica and Clarias lazera-during autumn, winter, spring and summer-as mean values of 3x10^3 and 2x10^3, 2x10^3 and 10^3, 8x10^3 and 4x10^3 besides 7x10^3 (each) organisms/g, respectively by **Darwish et al. (1991);** in identical samples of Clarias lazera and Tilapia nilotica-collected from fish markets-as mean of 6.84x10^3 and 3.72x10^3 organisms/g, successively by **Mousa and Mahmoud (1997);** in flesh of iced Tilapia nilotica as a range of 4.8x10^3-6x10^5

organisms/g by **Awad (1998)**; in correspondent samples of Tilapia nilotica and Clarias lazera as mean of 10^4 organisms/g, each by **Sheriff (1998)**; in Tilapia fish as a range of mean levels $2.6x10^3$-$2.1x10^5$ organisms/g by **Metawea and Abd El-Ghaffar (2007)**; in Tilapia nilotica and Mugil cephalus as mean of $2x10^4$ and $7x10^3$ organisms/g, consecutively by **Saleh and El-kewaiey (2008)**, successively.

Viewing the findings which described both occurrence and populations of Enterobacteriaceae organisms in tested fishes, obtained in this work, indicates the presence of these contaminants in surveyed fresh freshwater and frozen marine fishes by nearly equal and relatively high percentages, as well as by slightly lower contaminated samples than those identical ones harbored both aerobic mesophilic and psychrotrophic bacteria.

Relatively widespread occurrence of Enterobacteriaceae organisms in examined flesh samples agreed with the literature of Lerke et al. (1978) who emphasized that such bacteria in fish muscles are derived from the normal flora of skin, gills and intestine; besides the declaration of **Ogbondeminu and Okoeme (1989)** who reported that about 50% of the bacterial contaminants-recovered from both fishes and waters of earthen ponds fertilized with animal faecal waste-were members of Enterobacteriaceae in addition to both literatures of **Lindberg et al. (1998) and González-Rodriguez et al. (2001)** who assured that fishes can acquire the Enterobacteriaceae contaminants from their digestive tracts alongside aquatic and processing environments. Furthermore, the comparatively higher levels of Enterobacteriaceae organisms in surveyed flesh of freshly killed Clarias lazera than those detected in identical samples every of Tilapia nilotica and Mugil cephalus may be attributed to the higher intensities of such bacteria on the surface of non-scaly fishes (Clarias lazera) than those on scaly ones (Tilapia nilotica and Mugil cephalus) because the absence of scales giving a wide chance for these organisms to penetrate easily into the fish muscles **(Boulenger, 1907)**. Also, slightly higher Enterobacteriaceae counts in tested flesh samples of raw freshwater fishes than those recognized in the similar samples of frozen marine ones can be explained by the declaration of **Roberts (1978)** who attributed the higher populations of Enterobacteriaceae organisms in freshwater fishes than those in marine ones, to the habitat of the former types, because fresh water contains higher organic load than that found in sea water thus becomes more favourable for the survival of many bacteria particularly Enterobacteriaceae alongside the salinity of sea water inhibits the growth of such organisms.

With relation to the bacteriological techniques used for investigating the presence and populations of Staphylococcus aureus organisms in tested flesh samples, plates of Baired-Parker with egg-yolk tellurite emulsion alongside some laboratory tests could not find such organisms in all tested flesh samples (Tables 1&2). Similarly, **El-Gohary and Samaha (1992) as well as Ahmed and El-Khawas (2008)** could not find Staphylococcus aureus organisms in flesh samples of fresh Tilapia nilotica or frozen Salmon, respectively. On contrary, **Dorho (1998), Abo Samra (2001), Boari et al. (2008) and Saleh**

and El-Kewaiey (2008) could detect these bacteria in the correspondent samples of Tilapia nilotica, Mugil cephalus, Clarias lazera, fresh marine fishes, frozen Mackerel by a variety of occurrence and intensities. Additionally, absence of such bacteria in raw flesh samples every of tested freshwater and frozen marine fishes, could be explained by the literature of **Shewan (1871)** who assured that staphylococcal counts on freshly caught fishes very low.

Plates of starch-ampicillin agar-flooded with lugol iodine solution-showed the presence of Aeromonas-contamination in 7 (70%) samples by ranges (mean levels) of $0\text{-}4x10^2$ ($2x10^2\pm0.7xl0^2$) in iced Tilapia nilotica; 8 (80%) identical samples by $0\text{–}8x10^2$ ($3.6x10^2\pm0.9xl0^2$) in iced Mugil cephalus as well as in 6 (60%) similar samples by $0\text{–}9.7x10^2$ ($3.5x10^2\pm1.3xl0^2$) organisms/g in freshly killed Clarias lazera, respectively. Almost equal prevalence and populations of Aeromonas contaminants were detected in correspondent flesh samples of frozen marine fishes too, where they found in 9 (90%) samples by $0\text{–}9x10^2$ ($3.9x10^2\pm10^2$) in frozen Mackerel; 7 (70%) samples by $0\text{–}6.5x10^2$ ($3.1x10^2\pm0.7xl0^2$) in frozen Sardine in addition to 10 (100%) samples by $1.4x10^2\text{–}9x10^2$ ($4.2x10^2\pm0.9xl0^2$) organisms/g in frozen Herring, consecutively (Tables 1 & 2).

An equal incidence of Aeromonas organisms (90%) was obtained in fish samples by **Tsai and Chen (1996)** in Switzerland, whilst lower occurrence of these bacteria were evaluated by **Bastawrows and Mohammed (1999)** as 46% and 18% of Oreochromis niloticus and Clarias lazera samples; **Wang and Silva (1999)** as 36.1% of catfish fillets; Abo-El-Alla (2000) as 48% and 36.67% of Oreochromis niloticus and Labeo niloticus samples; **Davies et al. (2001)** as 40% of Eel, Mugil cephalus, Salmon, Mackerel and Sardine samples; **Thayumanavan et al. (2003)** as 37.3% of freshly caught finfishes; **Vivekanandhan et al. (2005)** as 33.58% of Mugil, Sardine and Mackerel fishes as well as by **Saleh and El-Kewaiey (2008)** as 32%, 40% and 16% of Tilapia nilotica, Mugil cephalus and frozen Mackerel fishes, respectively. Furthermore, approximately similar Aeromonas intensities were obtained by **Dorho (1998)** as a mean of $5x10^5$ in flesh samples of Mugil cephalus; alongside by **Boari et al. (2008)** as a mean of $1.5x10^2$ organisms/g in fresh Tilapia fillets. On the contrary, higher Aeromonas counts were detected in flesh samples of Tilapia nilotica as a mean of $8.9x10^2$ by **Dorho (1998)**; in Oreochromis niloticus and Clarias lazera flesh as mean levels of $3.4x10^4$ and $4.1x10^3$, consecutively by **Bastawrows and Mohammed (1999)**; in Oreochromis niloticus flesh as a mean of $3.2x10^2$ by **Abo-El-Alla (2000)** besides in Tilapia nilotica, Mugil cephalus and frozen Mackerel flesh as mean of $7x10^3$, $2x10^4$ and $4x10^3$ organisms/g, respectively by **Saleh and El-Kewaiey (2008)**.

Analysis of the obtained findings reveals the widespread contamination of tested fish samples with Aeromonas organisms; the fact that agree with literatures of **Monfort and Baleux (1990), Adams and Moss (2000) and Kirov (2003)** who declared that these bacteria constitute a group of ubiquitous microorganisms of aquatic environments like fresh water, coastal water and sewage alongside the psychrotrophic nature of most them particu-

larly A. hydrophila which can grow at cold temperatures up to zero °C for some strains. Also, higher percentages of Aeromonas-contaminated samples among surveyed Tilapia nilotica than those found in Clarias lazera fishes, can precisely explained by the literature of **Akelah (1978)** who attributed the existence of Aeromonas organisms in Oreochromis niloticus with higher prevalence and intensities than those found in Clarias lazera, to presence of them as normal inhabitant in the intestine of former fishes. Additionally, the relative increase of Aeromonas-contaminated samples among frozen fishes may give an indication about the resistance of such bacteria against freezing storage.

The exclusive testing of fresh freshwater fishes for presence of salmonellae in their flesh samples, plates of xylose lysine desoxycholate (XLD) agar alongside biochemical and serological tests could detect such organisms only in one (10%) sample from each type of these fishes, after being enriched in both buffered peptone water and Rappaport-Vassiliadis broth, (Tables 1). The obtained salmonellae were further serotyped as 3 strains of S. kottobus recovered from contaminated samples (one strain from each type) (Table 4).

By comparison, the findings of many workers agreed with the obtained results, where **Floyd and Jones (1954)** recovered 12 Salmonella strains from freshly caught freshwater fishes; **Naser (1991)** obtained salmonellae from 8% out of 25 Tilapia nilotica muscles; **Singh et al. (1992)** isolated S. paratyphi and other serotypes from the flesh of freshwater fishes; **Dorho (1998)** found S. typhimurium in Clarias lazera muscles; **Metawea and Abd El-Ghaffar (2007)** recognized salmonellae in 3.3-10% flesh samples of 60 Tilapia fishes as well as **David et al. (2009)** isolated S. typhi and other food poisoning salmonellae from the flesh of Nile Tilapia fishes. Additionally, several results went contrary to those obtained in the present study too, by many researchers where **Dorho (1998); Abo-El-Alla and Bastawrows (1999); Davies et al. (2001) and Boari et al. (2008)** could not recover salmonellae from flesh samples of raw freshwater fishes like Tilapia nilotica, Mugil cephalus, Clarias lazera, Oreochromis niloticus, Labeo niloticus and Eel besides gills and intestinal content of both Oreochromis niloticus and Labeo niloticus fishes. However, higher occurrence of Salmonella were found in 43.3% flesh samples of 101 Tilapia nilotica surveyed by **Youssef et al. (1992)**.

Explanation of the obtained findings may be found in the literatures of **Lawson (1970)** who assured that fishes did not normally suffer from Salmonella infections, however, they might mechanically carry these organisms if caught from polluted waters alongside the declaration of **Feldhusen (2000)** who attributed the presence of salmonellae in water of fish farms, to the pollution from a nearby poultry farms in addition to some Salmonella serovars may be found in fish feed and its ingredients. Additional source of fishes contamination with such bacteria is the ice used for refrigerating fishes, when made from polluted water **(Hobbs, 1954 and Falcão et al., 2002)**. Furthermore, the scarcity of Salmonella-contaminated samples among surveyed fishes agreed with those reported by **Nastasi et al. (1990)** who mentioned that Salmonella kottobus has been an unusual finding at

centers of Enterobacteriaceae in Italy; where among Salmonella strains examined by molecular genetics method between 1982 and 1985, only 0.7%, 0.8% and 0.1% isolates belonged to this serovar, in addition to **Blackburn and McClure (2002)** who stated that if salmonellae present in a food, it is likely to be present only at a low frequency.

Investigating the vibrios was solely performed in samples taken from marine fishes, where plates of thiosulfate citrate bile sucrose (TCBS) agar alongside the confirmatory tests show the occurrence of these bacteria only in one (10%) sample of frozen Mackerel, while this presence was exclusively found in 2 (20%) samples of frozen Sardine, after being enriched in alkaline peptone water. The same bacteriological techniques could not recover these contaminants from frozen Herring (Tables 1). A sum of obtained 3 Vibrio strains were further identified as 1 V. cholerae plus 2 V. parahaemolyticus strains; the former one was recovered from frozen Sardine, whereas the latter 2 strains were obtained from muscles of frozen Mackerel plus frozen Sardine (one strain from each) (Table 4).

By comparison, nearly similar results were obtained by several workers all over the world, where vibrios could be detected by **Baffone et al. (2000)** in 13.15% flesh samples of marine fishes and V. parahaemolyticus organisms were obtained in Greece by **Davies et al. (2001)** as a percentage of 14% overall Salmon, Mackerel plus Sardine samples. Meanwhile, higher percentage of V. parahaemolyticus-contaminated samples was estimated as 35% by **Davies et al. (2001)** in Portugal for the overall flesh samples of Salmon, Mackerel plus Sardine fishes as well as higher vibrios-contaminated flesh samples of Sardine were detected in Egypt as 80% by **Farag and Korashy (2007)**.

Detailed inspection of the obtained findings exhibits the very little presence of vibrios in surveyed fishes although the widespread occurrence of such organisms in sea water; where **ICMSF (1986), FAO/WHO (2001) and Cook et al. (2002)** stated that V. parahaemolyticus organisms present naturally in the marine environments and frequently isolated from a variety of seafoods comprised Sardine and Marckel particularly those taken from tropical or subtrobical trobical waters or from temperate-zone waters in summertime. However, such decreased occurrence in contaminated samples of frozen marine fishes agreed with the literature of **Shen et al. (2009)** who emphasized the disappearance of V. parahaemolyticus organisms in shocked Oysters after 60 days-storage at -18°C; the same degree of most commercial freezing temperatures, although **Ries et al. (1992)** assured that freezing does not kill V. cholerae organisms which can survive in ice for 4-5 weeks.

Microbiological risk assessment of all surveyed fishes, through comparing different bacterial populations contained in their tissues with the relevant limits, recommended by **ICMSF (1986); Gilbert et al. (2000) and PHLS (2000)**, showed that 3 (10%) out of the overall 30 fresh freshwater fish muscle samples were contaminated with Salmonellae and 1 (10%) out of 10 frozen Sardine muscle samples were harboured Vibrio cholerae organisms, thus exceeded the recommended limit (free for both organisms), whereas, none of surveyed fish samples contained aerobic mesophiles, Enterobacteriaceae or Staphylococ-

cus aureus organisms by intensities more than the recommended limits (10^7, $<10^4$ and $<10^4$ organisms/g, consecutively) (Table 3).

Data arranged in Table (4) exhibit the types and numbers of bacterial strains-obtained from the surveyed flesh samples of fresh freshwater and frozen marine fishes- after being picked up randomly from the separate colonies each of psychrotrophic grown onto plate count agar, presumptive Aeromonas spp on starch-ampicillin agar, presumptive Salmonella spp on XLD agar and Vibrios spp on TCBS agar, a total of 73 strains distributed as 2 strains of Acinetobacter calcoaceticus; 2 and 2 Aeromonas hydrophila and Aeromonas salmoncida; 2 Alcaligenes faecalis; 4 and 3 Bacillus cereus and Bacillus circulans; 1 Branhaemlla catarrhalis; 4 and 3 Chromobacterium lividum and Chromobacterium violaceum; 4 Citrobacter freundii; 2 Corynebacterium ulcerans; 2 and 5 Enterobacter agglomerans and Enterobacter cloacae; 3 Escherichia coli; 3 and 2 Micrococcus luteus and Micrococcus varians; 2 Niessera flavescencs; 1 Proteus vulgaris; 5, 3 and 4 Pseudomonas aeruginosa, Pseudomonas alcaligenes and Pseudomonas fluorescens; 3 Salmonella Kottbus; 1 Serratia liquefaciens; 4 and 3 Staphylococcus aureus and Staphylococcus epidermidis besides 1 and 2 strains of Vibrio cholerae and Vibrio parahaemolyticus, successively.

Typing of Enterobacteriaceae organisms-picked up randomly from the separate colonies grown onto plates of violet red bile glucose agar and obtained from flesh samples of all tested fresh freshwater and frozen marine fishes- showed a sum of 36 Enterobacteriaceae strains identified as 5, 1 and 1 strains of Citrobacter freundii, Citrobacter diversus and Citrobacter morganii; 4, 2 and 8 Enterobacter agglomerans, Enterobacter cloacae and Enterobacter hafniae; 4 Escherichia coli; 2, 1 and 2 Proteus mirabilis, Proteus rettgeri and Proteus vulgaris; 1 Provedencia alcalifaciens plus 3 and 2 strains of Serratia liquefaciens and Serratia rubidaea, consecutively (Table 5).

By comparison, **El-Gohary and Samaha (1992), Mousa and Mahmoud (1997), Sheriff (1998), Abo Samra (2001), Saleh and El-Kewaicy (2008), David et al. (2009), Farag and Korashy (2009) and Yagoub (2009)** could identify bacterial isolates, identical to most of those obtained in this work, as Acinetobacter spp, Aeromonas hydrophila, Alcaligenes spp, Citrobacter freundii, Citrobacter diversus, Enterobacter cloacae, Escherichia coli, Micrococcus spp, Proteus mirabilis, Proteus rettgeri, Proteus vulgaris, Provedencia alcalifaciens, Pseudomonas aeruginosa, Pseudomonas alcaligenes, Pseudomonas fluorescens, Salmonella spp, Serratia liquefaciens, Serratia rubidaea, Staphylococcus aureus, Staphylococcus epidermidis, recovered from the flesh, skin, gills and intestinal content of the prevalent freshwater fishes included Tilapia nilotica, Clarias lazera, Mugil cephalus, Oreochromis niloticus and Labeo niloticus. Similarly, some relevant bacterial strains-obtained also from marine fishes included Mackerel, Sardine, Tuna, Pugrus, Solea and Bonito-identified as Citrobacter freundii, Enterobacter agglomerans, Enterobacter cloacae, Escherichia coli, Proteus mirabilis, Proteus vulgaris and Pseudomonas fluorescens by **Abd El-Maksoud (1992), Shalaby (1992), Awad and Khalafalla (1993) and Sheriff (1998).**

Results

Table (1): Numbers and percentages of back- muscles of fishes, contaminated with different bacterial populations (n*=10 each).

Types of Examined fishes \ No. and % of contaminated muscles		*Aerobic mesophiles-contaminated muscles*	*Psychrotrophs-contaminated muscles*	*Enterobacteriaceae-contaminated muscles*	*Staphylococcus aureus-contaminated muscles*	*Aeromonas-contaminated muscles*	*Salmonella-contaminated muscles*	*Vibrio-contaminated muscles*
Freshwater fishes	Iced Tilapia nilotica	10 (100%)	10 (100%)	8 (80%)	0 (0%)	7 (70%)	1 (10%)	Not tested
	Iced Mugil cephalus	10 (100%)	10 (100%)	9 (90%)	0 (0%)	8 (80%)	1 (10%)	
	Freshly killed Clarias lazera	10 (100%)	10 (100%)	6 (60%)	0 (0%)	6 (60%)	1 (10%)	
Marine fishes	Frozen Mackerel	10 (100%)	10 (100%)	9 (90%)	0 (0%)	9 (90%)	Not tested	1 (10%)
	Frozen Sardine	10 (100%)	9 (90%)	6 (60%)	0 (0%)	7 (70%)		2 (20%)
	Frozen Herring	10 (100%)	8 (80%)	10 (100%)	0 (0%)	10 (100%)		0 (0%)

n* = number of examined samples.

Table (2): Intensities of bacterial populations per gram of back- muscles of whole fishes (n*=10 each).

Bacteriological techniques / Types of examined Muscles		*Aerobic plate counts*			*Psychrotrophic counts*			*Enterobacteriaceae counts*			*Staphylococcus aureus counts*			*Aeromonas counts*		
		Min	**Max**	**Mean ± SE**	**Min**	**Max**	**Mean ± SE**	**Min**	**Max**	**Mean ± SE**	**Min**	**Max**	**Mean ± SE**	**Min**	**Max**	**Mean ± SE**
Iced	**Tilapia nilotica**	3×10^2	1.3×10^5	2.8×10^4 $\pm1.3\times10^4$	1.1×10^3	8.9×10^4	3×10^4 $\pm10^4$	0	1.6×10^3	2.6×10^2 $\pm1.5\times10^2$	ND	ND	ND	0	4×10^2	2×10^2 $\pm0.7\times10^2$
	Mugil cephalus	10^2	1.4×10^5	2.6×10^4 $\pm1.4\times10^4$	1.1×10^3	9.3×10^4	2.3×10^4 $\pm10^4$	0	7×10^2	2.4×10^2 $\pm0.7\times10^2$	ND	ND	ND	0	8×10^2	3.6×10^2 $\pm0.9\times10^2$
Freshly killed Clarias lazera		2×10^2	1.5×10^5	4.6×10^4 $\pm2.3\times10^4$	10^2	8.8×10^4	1.2×10^4 $\pm0.9\times10^4$	0	4.2×10^3	7.1×10^2 $\pm2.4\times10^2$	ND	ND	ND	0	9.7×10^2	3.5×10^2 $\pm1.3\times10^2$
Frozen	**Mackerel**	4×10^2	1.9×10^4	5.3×10^3 $\pm1.9\times10^3$	10^2	2.1×10^4	3.6×10^3 $\pm2\times10^3$	0	3.7×10^3	4.3×10^2 $\pm1.6\times10^2$	ND	ND	ND	0	9×10^2	3.9×10^2 $\pm10^2$
	Sardine	2×10^2	3.2×10^4	6.4×10^3 $\pm3\times10^3$	0	3.2×10^4	4.7×10^3 $\pm3.1\times10^3$	0	2.9×10^2	8.3×10 $\pm3.4\times10$	ND	ND	ND	0	6.5×10^2	3.1×10^2 $\pm0.7\times10^2$
	Herring	1.2×10^3	2.4×10^5	6×10^4 $\pm2.6\times10^4$	0	1.4×10^3	4.7×10^2 $\pm1.3\times10^2$	2×10	2.5×10^2	1.1×10^2 $\pm0.2\times10^2$	ND	ND	ND	1.4×10^2	9×10^2	4.2×10^2 $\pm0.9\times10^2$

n*= number of examined samples. Min= minimum. Max= maximum. SE= standard error. ND= not detected

Table (3): Microbiological risk assessment of surveyed fishes, through comparing different bacterial populations contained in their tissues with the recommended limits.

Bacteriological findings in relation to limits / Types of examined samples	*Aerobic plate counts*		*Enterobacteriaceae counts*		*Staphylococcus aureus counts*		*Salmonella detection*		*Vibrio cholerae detection*	
	Recommended limits*	No. (%) of samples exceeding the limits	Recommended limits*	No. (%) of samples exceeding the limits	Recommended limit*	No. (%) of samples exceeding the limits	Recommended limits* in 25 g-sample	No. (%) of samples exceeding the limits	Recommended limits* in 25 g-sample	No. (%) of samples exceeding the limits
Raw fishes	$< 10^7$	0 (0%)	$< 10^4$	0 (0%)	$< 10^4$	0 (0%)	Free	3 (10 %) out of 30 fresh freshwater fish muscle samples	Free	1 (10%) out of 10 frozen sardine muscle samples

*= different recommended limits were reported by **ICMSF (1986); Gilbert et al. (2000)** and **PHLS (2000)**.

Table (4): Types and numbers of bacterial strains isolated from the muscles of fishes.

Types of fish samples / Types and numbers of bacterial strains	Iced whole		Freshly killed Whole Clarias lazera	Frozen whole		
	Tilapia nilotica	Mugil cephalus		Mackerel	Sardine	Herring
Acinetobacter calcoaceticus (2)	1	-	-	-	1	-
Aeromonas hydrophila (2)	-	1	-	1	-	-
Aeromonas salmoncida (2)	1	-	-	1	-	-
Alcaligenes faecalis (2)	1	-	1	-	-	-
Bacillus cereus (4)	-	-	-	2	-	2
Bacillus circulans (3)	-	-	-	-	2	1
Branhamella catarrhalis (1)	1	-	-	-	-	-
Chromobacterium lividum (4)	1	3	-	-	-	-
Chromobacterium violaceum (3)	1	-	-	2	-	-
Citrobacter freundii (4)	1	-	1	-	2	-
Corynebacterium ulcerans (2)	-	1	1	-	-	-
Enterobacter agglomerans (2)	-	1	-	-	-	1
Enterobacter cloacae (5)	1	2	-	-	-	2
Escherichia coli (3)	1	-	-	2	-	-
Micrococcus luteus (3)	1	1	-	1	-	-
Micrococcus varians (2)	-	1	-	1	-	-
Niesseria flavescencs (2)	1	1	-	-	-	-
Proteus vulgaris (1)	-	1	-	-	-	-
Pseudomonas aeruginosa (5)	-	1	2	1	1	-
Pseudomonas alcaligenes (3)	-	1	-	1	1	-
Pseudomonas fluorescens (4)	-	-	1	1	2	-
Salmonella kottbus (3)	1	1	1	-	-	-
Serratia liquefaciens (1)	1	-	-	-	-	-
Staphylococcus aureus (4)	1	-	1	1	-	1
Staphylococcus epidermidis (3)	-	-	1	1	1	-
Vibrio cholerae (1)	-	-	-	-	1	-
Vibrio parahaemolyticus (2)	-	-	-	1	1	-
Total strains = (73)*	14	15	9	16	12	7

* = these strains were randomly picked up from the psychrotrophs grown onto plate count agar, presumptive *Aeromonas* spp on starch-ampicillin agar, presumptive *Salmonella* spp on XLD agar and *Vibrio* spp on TCBS agar.

Table (5): Types and numbers of Enterobacteriaceae strains isolated from the muscles of fishes.

Types of fish samples / Types and numbers of Enterobacteriaceae strains	Iced whole		Freshly killed Whole Clarias lazera	Frozen whole		
	Tilapia nilotica	Mugil cephalus		Mackerel	Sardine	Herring
Citrobacter freundii (5)	1	1	2	1	-	-
Citrobacter diversus (1)	1	-	-	-	-	-
Citrobacter morganii (1)	1	-	-	-	-	-
Enterobacter agglomerans (4)	1	1	1	-	1	-
Enterobacter cloacae (2)	1	-	1	-	-	-
Enterobacter hafniae (8)	2	1	3	-	1	1
Escherichia coli (4)	2	-	2	-	-	-
Proteus mirabilis (2)	-	-	-	1	-	1
Proteus rettgeri (1)	-	-	1	-	-	-
Proteus vulgaris (2)	1	-	-	-	1	-
Providencia alcailfaciens (1)	1	-	-	-	-	-
Serratia liquefaciens (3)	1	1	1	-	-	-
Serratia rubidaea (2)	1	-	1	-	-	-
Total strains = (36)*	(13)	(4)	(12)	(2)	(3)	(2)

* = these obtained strains were randomly picked up from plates of violet red bile glucose agar.

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الملخص العربى

السمات الجراثيمية لأسماك المياه العذبة الطازجة والأسماك البحرية المجمدة بمدينة المنصورة

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تناولت الدراسة الفحص الجرثومى لعدد ستين عينة من الأسماك السليمة حسياً الأكثر استهلاكاً فى مصر وهى ثلاثون عينة من كل من أسماك المياه العذبة الطازجة (عشر عينات من كل من البلطى النيلى المحفوظ بالثلج، البورى الأصيل المحفوظ بالثلج والقرموط اللازير الميت حديثاً) وأسماك المياه المالحة المجمدة (عشر عينات من كل من الماكريل، السردين والرنجة) والتى تم شراؤها من الأسواق المنتشرة فى مدينة المنصورة وقد مثلت كل واحدة من الأسماك المختبرة بـ 60 جرام من عضلة الظهر والتى تم استئصالها تحت ظروف صحية معقمة، وقد أظهرت أطباق العد الجرثومى Plate count agar تواجد الجراثيم الهوائية المحبة للحرارة المعتدلة aerobic mesophiles فى جميع العينات المختبرة (100%) بمتوسطات $^{4}10x2.8$,$^{4}10x2.6$-,$^{4}10x4.6$ جرثوم/جرام من أسماك المياه العذبة الطازجة، فى حين كانت $^{4}10x6$,$^{3}10x6.4$,$^{3}10x5.3$ جرثوم/جرام من الأسماك المالحة المجمدة، على التوالى، وقد أظهرت أطباق العد الجرثومى plate count agar تواجد الجراثيم الصامدة للبرودة psychrotrophs فى 8(80%)-10 (100%) من نفس العينات بمتوسطات $^{4}10x3$,$^{4}10x2.3$,$^{4}10x1.2$ بالإضافة إلى $^{3}10x3.6$,$^{3}10x4.7$,$^{2}10x4.7$ جرثوم/جرام، فى حين تواجدت الجراثيم المعوية Enterobacteriaceae بنسب 6(60%) - 8(80%),9(90%) - 10 (100%) فى كلتا النوعين من الأسماك بمتوسطات $^{2}10x2.6$, $^{2}10x2.4$, $^{2}10x7.1$, $^{2}10x4.3$,$^{2}10x8.3$,10x1.1 جرثوم/جرام، على التوالى، بينما لم تواجدت جراثيم المكور العنقودى الذهبى Staphylococcus aureus فى أى من الأسماك المختبرة على الإطلاق، وقد أظهرت الاختبارات الجراثيمية تواجد جراثيم الزوائف الهوائية Aeromonas spp فى 6(60%)-8 (80%) من أسماك المياه العذبة الطازجة و 7 (70%) - 10 (100%) من أسماك المياه المالحة المجمدة بمتوسطات

$^{2}10x2$,$^{2}10x3.6$,$^{2}10x3.5$,$^{2}10x3.9$, $^{2}10x3.1$, $^{2}10x4.2$ جرثوم/ جرام، على الترتيب، وبالنسبة إلى جراثيم سالمونيلا Salmonella فقد تواجدت فى 1(10%) من كل من عينات البلطى النيلى المحفوظة بالثلج، البورى الأصيل المحفوظة بالثلج والقرموط اللازير الميت حديثاً، وكانت العترات الثلاثة المعزولة من نوع سالمونيلا كوتباس S.kottbus بينما تواجدت جراثيم فيبريو Vibrio spp فقط فى 1(10%) من الماكريل المجمد بالإضافة إلى 2(20%) من السردين المجمد والتى صنفت إلى عترة واحدة من الفيبريو كوليرا V.colerae عترتين من الفيبريو باراهيموليتيكاس V.parahaemolyticus.

وفيما يتعلق بتقييم الخطر الميكروبيولوجى المحتمل للأسماك المختبرة من خلال مقارنة التجمعات الجراثيمة الموجودة بأنسجتها مع تلك الحدود المسموح بها لكل نوع من هذه الجراثيم، فقد أسفر عن تجاوز 3(30%) من إجمالى 30 عينة من أسماك المياه العذبة الطازجة بالنسبة لتواجد الجراثيم سالمونيلا، إلى بجانب 1(10%) من 10 عينات من أسماك السردين المجمد بالنسبة لجراثيم فيبريو كوليرا عن الحد المسموح

به (عدم تواجد تلك الجراثيم على الإطلاق)، بينما لم تتخطى أى من عينات الأسماك المختبرة بالنسبة للجراثيم الهوائية المحبة للحرارة المعتدلة، الجراثيم المعوية وجراثيم المكور العنقودى الذهبى الحد المسموح به (أقل من $10^{7}10$, 10^{4}, 10^{4} جرثوم/جرام، على التوالى).

وقد تم أخذ ثلاثة وسبعين عترة من الأطباق الخاصة بكل من الجراثيم الصامدة للبرودة والزوائف الهوائية وسالمونيلا والفيبريو المعزولة من عينات الأسماك المختبرة لتصنيفها والذى أسفر عن تصنيف : 2 عترات اسنيتوباكتر كالكواستيكاس، 2,2 ايروموناس هيدروفيلا، وايروموناس سالمونسيدا، 2 الكاليجينس فيكاليس، 3,4 باسيلاس سيريس وباسيلاس سيركيولانس، 1 برانهيملا كاتاراليس،3,4 كروموباكتيريام ليفيدام وكروموباكتيريام فيولاشيام، 4 ستروباكتر فروندى، 2 كورينيباكتيريام السرانس، 5,2 انتيوباكتر اجلوميرانس وانتيروباكتر كلواكى، 3 ايشيريشيا كولاى، 2,3 ميكروكاكاس ليوتياس وميكروكوكاس فاريانس، 2 نيسيريا فلاقيسينس، 1 بروتيس فالجاريز، 4,3,5 سيدوموناس ايروجينوزا، سيدوموناس الكاليجينس وسيدوموناس فلوريسنس، 3 سالمونيلا كوتباس، 1 سيراتيا ليكويفيشانس، 4,3 تافيلوكوكاس أورياس وستافيلوكوكاس إبيدرميديس، بالإضافة إلى 2,1 عترات من فيبريو كوليرا و فيبريو باراهيموليتيكاس، على التوالى، ومن جهة أخرى تم أخذ عدد ست وثلاثون عتره من الأطباق الخاصة بالجراثيم المعوية من عينات الأسماك المختبرة لتصنيفها والذى أسفر عن تصنيف : 1,1,5 ستروباكتر فروندى، ستروباكتر دافيرساس وستروباكتر مورجانى 8,2,4 أنتيوباكتر أجلوميرانس، أنتيروباكتر كلواكى وأنتيروباكتر هافنيا، 4 ايشيريشيا كولاى 2,1,2 بروتيس ميرابيلس، بروتيس ريتيجرى وبروتيس فالجاريز، 1 بروفيدينشيا الكاليفاشيانس، بالإضافة إلى 2,3 سيراتيا ليكويفيشانس وسيراتيا روبيديا، على الترتيب.

هذا وقد تمت مناقشة الأهمية الصحية والاقتصادية للجراثيم المعزولة وكذلك النصائح الهادفة لمنع أو تقليل التلوث الجرثومى للأسماك المختبرة إلى أدنى مستوى له لضمان أقصى درجات الأمان الصحى للمستهلكين.