Bacteriological studies on Psychotropic bacteria and Pseudomonas isolated from frozen fish

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ABSTRACT

Frozen fish are exposed to many risks of contamination during long chain of catching, transportation, dressing and freezing from different sources. Till they reach to consumers which make it harmful or unfit for human consumption. A total of 100 random samples of frozen Saurus and Mackerel (50 of each) were collected from different fish markets at El –Menofia governorate. They were subjected to bacteriological examination for isolation of Psychotropic and Pseudomonas species. The bacteriological examination revealed that the mean values of total Psychotropic count in the examined samples of Saurus were $1.07 \times 10^3 \pm 2.82 \times 10^6$ and $1.14 \times 10^3 \pm 3.2 \times 10^6$ in Mackerel. The incidence of Pseudomonas species was 40% and 50% of the examined samples of Saurus and Mackerel, respectively. The incidence of identified Pseudomonas species isolated from the examined samples of frozen fish were Ps. aeruginosa, Ps. putida and Ps. fragi. The Pseudomonas species were resistant to chloramphenicol and nalidixic acid. In contrast they were sensitive to gentamycin except Ps. fluorescens.

1. INTRODUCTION

Fish has been regarded as a nutrition and highly desirable food due to its contribution of high-quality protein that we can easily and completely digest. It is very rich source of vitamins as vit B6, B12 and rich in mineral as Ca, Ph and iodine that are vital to our health. In addition, fishes are excellent source of unsaturated fatty acid which protect fish eater against heart diseases, obesity and hypertension. Bacterial contamination is either due to direct contamination of the fish by polluted water or due to secondary contamination during handling, processing, storage, preparation or distribution. Such contamination is important when fish is eaten raw or processed. Pseudomonas species are considered the most important Psychotropic microorganisms causing fish spoilage (Zayed-Amamy, 2004;Lu and Bi, 2007). Psychotropic bacteria are these bacteria that grow well at or below 7°C and have their optimum temperature for growth between 20-30°C. Pseudomonas species are opportunistic Gram- negative pathogens, normally occur in aquatic environment and as apart of normal gut flora of healthy fish. They cause outbreaks when normal environmental conditions were changed as high organic load, contaminated food, bad water quality and unhygienic conditions (Roberts, 2001). Common uses of antibiotics in food producing animals can result in antibiotic resistance of intestinal bacteria. Moreover, the resistance genes may be transferred to disease causing bacteria, resulting in antibiotic resistant infections for humans (Serrano, 2005).

Therefore, this work was planned out to study the presence of Psychotropic bacteria in frozen fish (Mackerel and Saurus) through estimation of Psychotropic counts, isolation and identification of Pseudomonas species in frozen fish. Plus, determination of their antibiotic susceptibility.

2. MATERIAL AND METHODS

2.1. Collection of fish samples:
A total of 100 random samples of frozen Saurus and Mackerel (50 of each) were collected from different fish markets in El-Menofia governorate to be examined bacteriologically for determination of Psychotropic and Pseudomonas isolation in such examined samples.

2.2. Preparation of samples (AOAC, 1990):
Frozen fish samples were left to thaw (2-5°C). Under complete aseptic conditions 10 gm of the back muscle was transferred into sterile homogenizer jar containing 90 ml of sterile 0.1% peptone water. The contents were homogenized for 2.5 minutes at room temperature (20°C) and then allowed to stand for 5 minutes. One ml of homogenate was transferred into sterile test tube contained 9ml of 0.1% peptone water to prepare a $10^6$ fold serial dilution up to $10^9$.

2.2.1. Determination of Psychotropic count (Collins and Lynne, 1984):

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From each dilution, 1 ml of homogenate was transferred by using a sterile pipette into two separate petri-dishes to which approximately 15 ml of sterile melted and tempered plate count agar (45°C) were added and mixed. The inoculated plates were gently shaken in rotary movement and left till complete solidification of the agar. The plates were inverted and incubated at 37°C for 10 days. The total Psychrotrophic count/g were calculated on plates containing 30-300 colonies.

2.2.2. Isolation and Identification of Pseudomonas:
The suspected colonies were purified and sub-cultured onto nutrient agar slopes and incubated at 37°C for 24 hrs. The purified colonies were subjected for further identification either microbiologically or morphologically (Gram stain, Motility test or biochemically according to Krieg and Holt (1984).

2.2.3. Antibiotic susceptibility test (Disc Diffusion) of Pseudomonas isolates:
Pseudomonas isolates were identified as sensitive, intermediate or resistant according to the National Committee for clinical laboratory standards Institute (CLSI) recommendations (Schreckenberger and Binnicker, 2011). Samples were processed for culture and sensitivity pattern for Pseudomonas was determined against commonly used antibiotics by disc diffusion method acc.to Benson, H.J. (1990). Muller-Hinton agar (CM0337-OXOID) was prepared according to manufacturer’s instructions and sterilized by autoclaving at 121°C for 15 min. Sterilized medium was then cooled in a water bath, and about 25 ml of medium was poured into 90 mm diameter sterile Petri-plates to a depth of 4 mm on a level surface to make the depth of the medium uniform and left at room temperature overnight to check sterility. Pseudomonas colonies were picked up (using a sterile loop) to a tube containing 5 ml of Muller-Hinton agar broth. The broth culture was incubated at 37°C for 24 hours. Then the turbidity was adjusted to the turbidity of the 0.5 McFarland standards. After that about 1 ml of the broth was inoculated on the surface of Muller-Hinton agar and spread evenly over the entire surface of the agar plates by a sterile bended glass rod. The antibiotic discs of Amoxicillin, Ampicillin, Penicillin, Chloramphenicol, Neomycin, Erythromycin, Streptomycin, kanamycin and Nalidixic acid, Sulfamethoxazole, Gentamycin, Ciprofloxacin, Norfloxacin and Oxytetracycline were applied on the inoculated plates. Then, the plates were placed in an incubator at 37°C for 18 h in inverted position. After 18 h of incubation, plates were examined and the diameters of zone of inhibition were measured in mm.

3. RESULTS

The total Psychrotrophic count/cfu/g of the examined frozen fish varied from 5.1 x 10⁶ to 7.1x 10⁷ with an average 1.07 x 10⁷ ± 2.82 x 10⁶ in Saurus and 2.1 x 10⁷ to 8.4 x 10⁷ with an average 1.14 x 10⁷ ± 3.2 x 10⁶ in Mackerel, respectively (Table 1). The incidence of Pseudomonas species isolated from the examined samples was 40% and 50% in Saurus and Mackerel, respectively (Table 2). The identified Pseudomonas species were Ps. aeruginosa, Ps. diminuta, Ps. fluorescence, Ps. putida and Ps. fragi (Table 3).

Results given in table (4) revealed that the Pseudomonas species isolated from the examined frozen fish were resistant to Chloramphenicol and Naldixic acid. In contrast they were sensitive to Gentamycin except Ps. fluorescence. Ciprofloxacin was drug of choice for Ps. aeruginosa infection. Ps. aeruginosa and Ps. fragi were sensitive to Ciprofloxacin. Ps. aeruginosa was intermediate sensitive to kanamycin, Amoxicillin and Norfloxacin. Ps. fragi was sensitive to Ampicillin, Streptomycin and Sulfamethoxazole. Meanwhile, Ps. putidawas sensitive to Neomycin and intermediate sensitive to Ciprofloxacin, Norfloxacin and Oxytetracycline. Ps. fluorescence was resistant to Ciprofloxacin, Gentamycin and kanamycin. Ps. diminuta was the only one sensitive to Kanamycin.

4. DISCUSSION

The Psychrotropic bacteria have received an increased attention by several investigators during recent years. The modern developments in fish and fish products have resulted in storage of fish for long period at low temperature which greatly slows the multiplication of bacteria, but not stops their growth, providing the favorable conditions for growth of Psychrotropic bacteria. It is evident from the result recorded in table (1) that the results of Psychrotrophic bacteria (cfu/g) in the examined samples were varied from 2.1 x 10⁶ to 8.4 x 10⁷ with an average 1.14 x 10⁷ ± 3.2 x 10⁶ in Mackerel; 5.1 x 10⁷ to 7.1 x 10⁷ with an average of 1.07 x 10⁷ ± 2.82 x 10⁶ in Saurus, respectively. These results came in accordance with those reported by El-Shafey (2014) (4.08 x 10⁷ ± 0.71 x 10⁷ cfu/g in Saurus, 9.95 x 10⁵ ± 2.13 x 10⁴ cfu/g in Mackerel, On the other hands, lower results obtained by Hassanean-Nermeen (2006), who found that the mean values of Psychrotrophic bacteria count in imported Mackerel were 4.6 x 10⁷ ± 1.2 x 10⁴ cfu/g. Higher results of Psychrotrophic count were obtained by El-Noby (2002) 2.4 x 10⁷ ± 8.2 x 10⁴ cfu/g in frozen Mackerel.

| Table 1 | Mean values of total Psychrotrophic bacterial counts of the examined frozen fish samples (n=50) of each |
|----------|--------------------------------------------------------|----------------|----------------|
| Fish Type | Min. | Max. | Mean± S.E. |
| Saurus | 5.1 x 10⁶ | 7.1 x 10⁷ | 1.07 x 10⁷ ± 2.82 x 10⁶ |
| Mackerel | 2.1 x 10⁷ | 8.4 x 10⁷ | 1.14 x 10⁷ ± 3.2 x 10⁶ |

S.E* = standard error of mean. ++ = High significant difference

| Table 2 | Incidence of total Pseudomonas species isolated of frozen fish samples (n=50 of each) |
|---------|----------------------------------|----------------|----------------|
| Fish type | NO. | % |
| Saur us | 20 | 40% |
| Mackerel | 25 | 50% |
| Total (100) | 45 | 45% |

| Table 3 | Incidence of Pseudomonas serotypes isolated from the examined frozen fish samples (n=50 of each) |
|---------|-----------------------------------------------------------|----------------|----------------|
| Pseudomonas Species | Saur us | Mackerel |
| Ps. aeruginosa | 7 | 15.5% | 9 | 18% |
| Ps. diminuta | 7 | 15.5% | 8 | 16% |
| Ps. fluorescence | 20 | 44.4% | 22 | 44% |
| Ps. putida | 5 | 11.1% | 6 | 12% |
| Ps. fragi | 6 | 13.3% | 5 | 10% |
| Total (100) | 45 | 45% | 50 | 50% |

Table 4 Results of the sensitivity tests for the Pseudomonas strains isolated from the examined frozen fish samples (n=25 of each).
The most Psychotropic bacteria contaminated these examined samples were Pseudomonas species. Nearly similar results were obtained by Yagoub (2009), who examined 150 fish samples and isolated Pseudomonas species from 62% of such samples.

Results showed that the highest incidence of Psychotropic bacteria were in Mackerel and the lowest in Saurus and this may be due to many sources of fish contamination as Workers boxes, boats, transportation under bad hygienic condition and bad freezing all these factors make fish lose its quality and cause public health hazard.

Results in table (2) illustrated that the incidence of Pseudomonas species isolated were 25 (50%) in Mackerel and in Saurus 20 (40%).

Higher results were obtained by EL-Shafey (2014), who isolated 76.67% from frozen Saurus and 66.67% from Mackerel, respectively. Lower results were obtained by Abu El-Atta (2003), who recorded that Ps. species were 26.05%. The incidence of Pseudomonas serotypes isolated from the examined samples of frozen fish in table (3) detected that, Ps. aeruginosa, Ps. diminuta, Ps. fluorescens, Ps. putida and Ps. fragi were isolated from 7 (15.5%), 7 (15.5%), 20 (44.4%), 5 (11.11%), 6 (13.3%) of Saurus 9 (18%), 8 (16%), 22 (44%), 6 (12%) and 5 (10%) of Mackerel samples, respectively.

Moreover, most examined samples of frozen fish were highly contaminated by Ps. fluorescens, followed by Ps. aeruginosa and Ps. diminuta. Nearly similar percentages were recorded by EL-Shafey (2014) that Ps. fluorescens was recovered in a rate of 16 (53.33%), 14 (46.67%) from Saurus and Mackerel, respectively. Higher results reported by Masbouha-Iman (2004), who isolated Pseudomonas from 103 fish samples. Lower results were obtained by El-Nagar (2010), who isolated Ps. fluorescens in a rate 34.6% from the examined fish.

Results given in table (4) revealed that the Pseudomonas species isolated from the examined samples of frozen fish were resistant to Chloramphenicol and Nalidixic acid. Ps. aeruginosa were sensitive to ciprofloxacin and Gentamycin.'Ps. aeruginosa' were intermediate resistant to Amoxicillin, Ampicillin and Ampicillin and sensitive to Amoxicillin and Ampicillin.

Penicillin and Streptomycin. P. fluorescens resists Gentamycin, Ciprofloxacinin and Chloramphenicol and nearly results recorded by Morgan (2014).

P. diminuta was sensitive to Gentamycin and Kanamycin, intermediate sensitive to Oxytetracycline and resist Neomycin, Ciprofloxacin, Ampicillin and Amoxicillin and nearly results recorded by Almazur et al. (2012).

Results detected that Ps. putida was intermediate sensitive to Ciprofloxacin, Norfloxacin and Oxytetracycline. Ps. putida was sensitive to Gentamycin and Neomycin and nearly results reported by Muller et al. (2011).

Results also detected that Ps. putida was resistant to Amoxicillin, Ampicillin, Erythromycin and Nalidixic acid and nearly results obtained by Espinosa et al. (2002).

Results shown that Ps. fragi was intermediate sensitive to Ampicillin and sensitive to Ampicillin, Sulfamethoxazole and Gentamycin. Ps. fragi was resistant to Oxytetracycline, Erythromycin and Chloramphenicol. Antibiotic resistance strains were occurred due to contamination of the frozen fish by polluted water or due to secondary contamination during handling, processing, and distribution subsequently Pseudomonas isolates acquired Antibiotic resistance.

5. CONCLUSIONS

From the obtained results it was concluded that the most Psychotropic bacteria contaminated these examined samples were Pseudomonas species. And these bacteria consider a major factor for the spoilage of fish or be a health hazard. The highest incidence of Pseudomonas species isolated from the examined frozen fish was in Mackerel then in Saurus. The most examined samples of frozen fish were highly contaminated by Ps. fluorescens, followed by Ps. aeruginosa. The Pseudomonas species isolated from the examined samples of frozen fish were resistant to chloramphenicol and Nalidixic acid.

6. REFERENCES


