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Bacteriological studies on *Psychotropic* bacteria and *Pseudomonas* isolated from frozen fish

Ashraf A. Abd El Tawab[1], Fatma I. EL-Hofy[1], Enas Soliman[1], Shaimaa M. Nada[2], Ahmed Osama[3,*]

[1]*Department of Bacteriology,* Immunology and Mycology, *Faculty of Veterinary Medicine Benha University, Egypt*
[2]*Department of food hygiene, Animal Health Research Institute , Shibin El kom, Egypt*

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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$1.07\times10^7\pm2.82\times10^6$ and $1.14\times10^7\pm3.2\times10^6$in Mackerel. The incidence of *Pseudomonas* species was 40%and50% 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. dimenuta, Ps. fluorescence, Ps. putida and Ps. fragi.* The *Pseudomonas* species were resistant to chloramphenicol and nalidixic acid. In contrast they were sensitive to gentamycin except *Ps. fluorescence*

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-Amany, 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 condtions10 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^{th} fold serial dilution up to 10^6.

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

* Corresponding author: **Ashraf A. Abd El Tawab.** *Department of Bacteriology,* Immunology and Mycology, *Faculty of Veterinary Medicine Benha University, Egypt*

From each dilution, 1ml of homogenate was transferred by using a sterile pipette into two separate petri-dishes to which approximately 15ml of sterile melted and tempered plate count agar (45°C) were added and mixed. The inoculated plates were gently shaken in rotatory movement and left till complete solidification of the agar. The plates were inverted and inoculated at (25°C) for 10 days. The total *Psychotropic* 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 24hrs. 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. (1998). 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 1ml 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 Oxytetracyclinewere 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 *Psychotropic* count/cfu/g of the examined frozen fish varied from 5.1×10^4 to 7.1×10^7 with an average $1.07\times10^7\pm 2.82\times10^6$ in Saurus and 2.1×10^4 to 8.4×10^7 with an average $1.14\times10^7\pm 3.2\times10^6$ 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* specieswere *Ps. aeruginosa, Ps. dimenuta, Ps. fluorescence, Ps. putida and Ps. fragi* (table 3).

Results given in table (4) revealed that the *Pseudomonas* species isolated from the examined frozen fishwere resistant to Chloramphenicol and Nalidixic 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. putida*was sensitive to Neomycin and intermediate sensitive to Ciprofloxacin, Norfloxacin and Oxytetracycline. *Ps. fluorescence* was resistant to Ciprofloxacin, Gentamycinand kanamycin. *Ps. dimenuta* was the only one sensitive to Kanamycin.

4. DISCUSSION

The *Psychotropic* 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 *Psychotropic* bacteria.It is evident from the result recorded in table(1) that the results of *Psychotropic* bacteria (cfu/g) in the examined samples were varied from 2.1×10^4 to 8.4×10^7 with an average $1.14\times10^7\pm 3.2\times10^6$ in Mackerel; 5.1×10^4 to 7.1×10^7 with an average of $1.07\times10^7\pm 2.82\times10^6$ in Saurus, respectively. These results came in accordance with those reported by El-Shafey (2014) ($4.08 \times 10^5 \pm 0.71\times 10^5$cfu/g in Saurus, $9.95 \times 10^5 \pm 2.13 \times 10^4$ cfu/g in Mackerel.On the other hands, lower results obtained by Hassaneen-Nermeen (2006), who found that the mean values of *Psychotropic* bacteria count in imported Mackerel were $4.6\times10^3\pm1.2\times10^2$ cfu/g. Higher results of *Psychotropic* count were obtained by El-Noby (2002) $2.4 \times10^{10} \pm 8.2\times10^6$ cfu/g in frozen Mackerel.

Table 1Mean values of total *Psychotropic* bacterial counts of the examined frozen fish samples (n=50) of each

Fish Type	Min.	Max.	Mean± S.E
Saurus	5.1×10^4	7.1×10^7	$1.07\times10^7\pm2.82\times10^6$
Mackerel	2.1×10^4	8.4×10^7	$1.14\times10^7\pm3.2\times10^6$

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	
	No	%	No	%
Ps. aeruginosa	7	15.5%	9	18%
Ps. diminuta	7	15.5%	8	16%
Ps. fluorescence	20	44.4%	22	44%
Ps. putida	5	11.11%	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).

Antimicrobial agent			Diffusion Zone (mm)	*Ps.fluorescence*	*Ps.aeruginosa*	*Ps.putida*	*Ps.diminuta*	*Ps.fragi*
Name	Symbol	Conc.(mg)						
Amoxicillin	AMX	30	≥ 14	7(R)	14(I)	11(R)	4 (R)	17(S)
Ampicillin	AM	10	≥ 13	3(R)	9(R)	7(R)	10(R)	16(S)
Chloramphenicol	C	30	≥ 12	8(R)	5(R)	1(R)	2(R)	5(R)
Ciprofloxacin	CP	5	≥ 15	11(R)	19(S)	16(I)	9(R)	17(S)
Erythromycin	E	15	≥ 13	1(R)	4(R)	7(R)	1(R)	6(R)
Gentamycin	GM	10	≥ 12	10(R)	22(S)	19(S)	15(S)	14(S)
Kanamycin	K	30	≥ 13	4(R)	14(I)	5(R)	20(S)	7(R)
Nalidixic acid	NA	30	≥ 13	6(R)	10(R)	9(R)	9(R)	3(R)
Neomycin	N	30	≥ 12	10(R)	11(R)	15(S)	4(R)	14(S)
Norfloxacin	NOR	10	≥ 12	4(R)	15(I)	12(I)	10(R)	2(R)
Oxytetracycline	T	30	≥ 14	11(R)	9(R)	14(I)	5(R)	8(R)
Penicillin	P	10 IU	≥ 20	22(S)	7(R)	14(R)	8(R)	15(R)
Streptomycin	S	10	≥ 11	12(S)	6(R)	1(R)	1(R)	13(S)
Sulfamethoxazole	SXT	25	≥ 10	7(R)	2(R)	5(R)	3(R)	14(S)

The most *Psychotropic* bacteria contaminated these examined samples were *Pseudomonas* species. Nearly similar results were obtained by Yagoub (2009), who examined150 fish samples and isolated *Pseudomonas* species from 62% of such samples.

Results shown 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 loss 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. dimenuta, Ps. fluorescence, 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. fluorescence*, followed by *Ps. aeruginosa* and *Ps. dimenuta*. Nearly similar percentages were recorded by, EL-Shafey (2014) that *Ps. fluorescence* was recovered in a rate of 16 (53.33%), 14 (46.67%) from Saurus and Mackerel, respectively. Higher results reported by Masbouba-Iman (2004), who isolated *Pseudomonas* from 103 fish samples. Lower results were obtained by El- Nagar (2010), who isolated *Ps. fluorescence* 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. Nearly results were recorded by Gales et al. (2001), who reported that Ciprofloxacin has been stated to be the most potent drug available for the treatment of *P. aeruginosa* infections.*Ps. aeruginosa* were intermediate resistant to Amoxicillin, Kanamycin and Norfloxacin.

Results detected that *P. fluorescence* was intermediate sensitive to Erythromycin and Ampicillin and sensitive to Penicillin and Streptomycin. *P. fluorescence* resists Gentamycin, Ciprofloxacinand 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 Almuzara 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. CONCULOSION

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. fluorescence*, followed by *Ps. aeruoginosa*. The *Pseudomonas* species isolated from the examined samples of frozen fish were resistant to chloramphenicol and Nalidixic acid.

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