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# Research Article
# Laboratory Screening of Biogenic Amines Producing Bacteria Potentially Threatens Human Health in Some Egyptian Fish and Fish Products

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## Abstract

**Background and Objective:** Fish and other fish products may be a causal agent of foodborne illnesses in humans if they contaminated by pathogenic bacteria, parasites, biogenic amines and toxins. The aim of this study was to screen some biogenic amines and their relation to microbial isolation in fish and fish products from the Egyptian markets. **Materials and Methods:** A total of 200 samples of fresh *Mugil cephalus*, smoked herring (*Clupea harengus*), fesikh (salted *Mugil cephalus*) and moloha (salted *Hydrocynus forskahlii*); 50 samples for each were collected randomly from markets and supermarkets of different localities in Egypt. **Results:** The bacteriological examination of tested samples revealed 207 bacterial isolates and were identified by using API strips. The most frequently isolated species were *Vibrio anguillarum* (17.4%), *Micrococcus* spp. (15.5%), *Escherichia coli* (12.1%), *Enterobacter aerogenes* (10.1%) and *Aeromonas hydrophila* (9.2%), while *Pseudomonas fluorescens*, *Clostridium perfringens* and *Proteus mirabilis* were isolated with low incidence (7.7, 6.8 and 6.3%, respectively). On the other hand *Staphylococcus xylosus* and *Streptococcus uberis* were isolated with very low incidence (3.4 and 1.4%). Screening of bacterial isolates demonstrated that 92 bacterial isolates (44.4%) were positive for biogenic amines production. From these, 43 (46.7%), 36 (39.1%) and 13 (14.2%) bacterial isolates were positive for cadaverine, putrescine and histamine, respectively. Some of these bacteria can cause serious diseases in fish and are often associated with human food borne disease. **Conclusion:** The application of early detection of biogenic amines producing bacteria and improvement of hygienic practices could reduce the risk of food poisoning.

Key words: Fish and fish products, bacterial isolates, API, biogenic amines

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

## INTRODUCTION

The consumption of fish and other seafood is increasing rapidly because fish contains easily digestible proteins and rich with polyunsaturated fatty acids. In recent years, more concerns about food safety, together with the consumer's demand for safe and healthier products have promoted studies of compounds with harmful effects on human health. The presence of pathogenic bacteria, parasites, biogenic amines and toxins may be a causal agent of foodborne illnesses in humans, range from mild gastroenteritis to life-threatening syndromes. These risk factors cannot be eliminated by thermal treatments, salting or freezing[1]. Some bacterial species facultatively pathogenic for both fish and man and may be isolated from fish without apparent symptoms of disease. Seafood is responsible for an important proportion of food-borne illness and outbreaks both in the United States and world wide, thus, biogenic amines and bacteria forming biogenic amines in fish and fish products has received considerable interest[2,3].

Biogenic amines are organic molecules of low molecular weight, which occur in a wide variety of foods, such as fish, fish products, meat, dairy products, wine and other fermented foods. Biogenic amines are organic bases with biological activity that are formed in fish and fish products by microbial decarboxylation of the corresponding amino acids or by transamination of aldehydes and ketones by amino acid transaminases[4,5]. The most important biogenic amines; histamine, tyramine, putrescine and cadaverine are originated from the decarboxylation of free amino acids namely histidine, tyrosine, ornithine and lysine, respectively. Putrescine, cadaverine, spermidine and spermine have an aliphatic structure; histamine and tryptamine have a heterocyclic structure and tyramine have an aromatic structure[6,7]. Consumption of foods containing high amounts of these amines can have toxicological effects. Alimentary administration of histamine can cause an allergy like syndrom, but the intake of high histamine causes histamine poisoning with symptoms including headaches, low blood pressure, broncho spasm, tachycardia, edema, vomiting, diarrhea and urticaria[8-10]. Also, putrescine, spermine, spermidine and cadaverine potentiate the toxicity of histamine and react with nitrite to form carcinogenic nitrosoamines[11,12].

Numerous strains of *Bacillus*, *Citrobacter*, *Clostridium*, *Klebsiella*, *Escherichia*, *Hafnia*, *Proteus*, *Pseudomonas*, *Salmonella*, *Shigella*, *Photobacterium*, *Lactobacillus*, *Pediococcus*, *Streptococcus*, *Enterococcus* and *Vibrio* have the ability to decarboxylate one or more amino acid[13]. They are bacterial species facultatively pathogenic for both fish and man and may be isolated from fish without apparent symptoms of disease[2]. Therefore, this study was planned to give additional scientific data on some biogenic amines and its relation to microbial isolation in fish and fish products from fresh *Mugil cephalus*, smoked herring, fesikh and moloha respectively from the Egyptian markets and supermarkets. Detecting histidine, lysine and ornithine decarboxylase activity by growth onto Niven's and modified Niven's agar media.

## MATERIALS AND METHODS

**Samples:** A total of 200 random samples of fish and fish products were purchased from markets and supermarkets of different localities in Cairo, Giza, Monofia and Qalupia Governorates. Fish samples included: Fresh *Mugil cephalus* fish (50), smoked herring (*Clupea harengus*) (50), fesikh (salted *Mugil cephalus*) (50) and moloha (salted *Hydrocynus forskahlii*) (50). Samples were transferred to the laboratory in ice box under complete aseptic conditions without dely and within 6 h. All representative samples were taken from muscle, homogenized by blender and immediately subjected to bacteriological and biogenic amines analysis.

**Bacteriological examination:** Tissue aliquots of 10 g from each fish sample were aseptically homogenized in a sterile homogenizer flask with 90 mL of sterile 0.1% peptone for 2 min. Specific media were used such as TCBS for isolation of *Vibrio* spp., violet red bile glucose agar and MacConkey agar with crystal violet for various Enterobacteriaceae, cephaloridine cetrimide fucidin agar for *Pseudomonas* and reinforced clostridial agar medium for anaerobic *Clostridia*. Pure cultures of the isolates were identified by biochemical characterization and further identification of each strains was achieved using the commercial API 20E, API 20NE, API for *Staphylococcus* API for *Streptococcus* (Biomérieux, France) according to manufacturer's instructions.

**Histidine decarboxylase activity:** Confirmation of histamine production by a microorganism was established by observing its ability to grow in Niven's medium[14] and Niven's modified medium[15]. It was considered that a strain was capable to produce histamine if test was positive. The tested strains were inoculated into 10 mL Tryptic Soy Broth (TSB, Difco) enriched with 1% histidine hydrochloride and incubated at 30°C for 24/48 h. The strain was then isolated on Tryptic Soy Agar (TSA, Difco) with 0.1% histidine, followed by incubation at 37°C for 24 h. Colonies were isolated and inoculated onto Niven medium and incubated at 30 and 37°C for 72 h, observing after every 24 h, a red colony, surrounded by a purple

ring indicated a positive reaction. Some microorganisms cannot grow on Niven's medium, because of its low pH (5.3). Yoshinaga and Frank[15] developed a medium that eliminated this problem which was also used. Composition of that medium was: Tryptone (0.5%), yeast extract (0.5%) and histidine (2%) at pH 6.5. This media was then sterilized at 121°C for 15 min. The preparation of the strains, their inoculation, incubation, etc., were carried out as in Niven's medium. A positive result was indicated by red colonies surrounded by a deep red ring.

**Confirmation of putrescine and cadaverine production:** Strains were inoculated in Muller broth for amino acids decarboxylation, supplemented with 1% of L- lysine and 1% of L-ornithine. Tubes were incubated at 30°C for 1-4 days. Samples were evaluated for the 2 amines (cadaverine and putrescine).

## RESULTS

**Isolation of bacteria potentially threatens human health:** A total of 207 bacterial isolates were isolated and identified by using API strips. The most frequently isolated species were *Vibrio anguillarum* (17.4%), *Micrococcus* spp. (15.5%), *E. coli* (12.1%), *Enterobacter aerogenes* (10.1%) and *Aeromonas hydrophila* (9.2%).

*Pseudomonas fluorescens*, *Clostridium perfringens* and *Proteus mirabilis* were isolated with low incidence (7.7, 6.8 and 6.3%, respectively). On the other hand *Staphylococcus xylosus* and *Streptococcus uberis* were isolated with very low incidence (3.4 and 1.4%) (Table 1).

**Biogenic amines production:** Screening of previous bacterial isolates by Niven's and modified Niven's agar demonstrated that 92 isolates (44.4%) were positive for biogenic amines production. From these, 43 (46.7%), 36 (39.1%) and 13 (14.2%) bacterial isolates were positive for cadaverine, putrescine and histamine, respectively.

Bacterial isolates that showed positive reaction onto Niven's medium and modified Niven's agar supplemented with lysine, ornithine and histidine to give biogenic amines (cadaverine, putrescine and histamine, respectively) were demonstrated as follow: both *Enterobacter aerogenes* and *E. coli* were the highest isolates that showed positive reaction for biogenic amines (80.9 and 80.0%) followed by *Pseudomonas fluorescens* (75%), *Citrobacter freundii* (60%) and *Proteus vulgaris* (54.5%) while *Micrococcus* spp., isolates showed positive reaction with an incidence of 37.5%.

*A. hydrophila* and *Vibrio anguillarum* showed positive reaction with an incidence of 25%. On the other hand the lowest bacterial isolates that showed positive reaction were *C. perfringens* and *S. xylosus* with an incidence of 21.4 and 14.2%, respectively.

## DISCUSSION

Foodborne disease is an important issue. Some bacterial species can cause serious diseases in fish. These bacteria and other found in fish and fish products are often associated with human diseases. In this study the most frequently isolated species were *Vibrio anguillarum*, *E. coli*, *Enterobacter aerogenes* and *Aeromonas hydrophila*, while *Pseudomonas fluorescens* and *Proteus mirabilis* were isolated with low incidence. These findings were agreed with previous studies in that the most microbial populations of stored fish almost consisted of gram-negative psychrotrophic bacteria, Enterobacteriaceae, *Pseudomonas* spp., and $H_2S$-producing bacteria[16-18].

Table 1: Frequency distribution of bacterial isolates in some fish and fish products as single and mixed organisms with percentage of biogenic amines producing isolates

| | Fresh Mugil | | Smoked herring | | Salted fesikh | | Salted moloha | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Bacteria | No. | (%) | No. | (%) | No. | (%) | No. | (%) | Total isolates | Biogenic amines producing isolates |
| *Vibrio anguillarum* | 11 | 18.6 | 8 | 17.0 | 10 | 16.7 | 7 | 17.1 | 36 (17.4%) | 9 (25%) |
| *Micrococcus* spp. | 10 | 16.9 | 7 | 14.8 | 8 | 13.3 | 7 | 17.1 | 32 (15.5%) | 12 (37.5%) |
| *E. coli* | 8 | 13.5 | 5 | 10.6 | 7 | 11.7 | 5 | 12.2 | 25 (12.1%) | 20 (80%) |
| *Aeromonas hydrophila* | 7 | 11.9 | 4 | 8.5 | 6 | 10.0 | 2 | 4.8 | 19 (9.2%) | 9 (25%) |
| *Enterobacter aerogenes* | 5 | 8.5 | 6 | 12.8 | 6 | 10.0 | 4 | 9.7 | 21 (10.1%) | 17 (80.9%) |
| *P. fluorescens* | 6 | 10.2 | 3 | 6.4 | 5 | 8.3 | 2 | 4.8 | 16 (7.7%) | 12 (75%) |
| *C. perfringens* | 3 | 5.1 | 2 | 4.3 | 5 | 8.3 | 4 | 9.7 | 14 (6.8%) | 3 (21.4%) |
| *Proteus mirabilis* | 5 | 8.5 | 1 | 2.1 | 4 | 6.7 | 3 | 7.3 | 13 (6.3%) | - |
| *Proteus vulgaris* | 2 | 3.4 | 2 | 4.3 | 3 | 5.0 | 4 | 9.7 | 11 (5.3%) | 6 (54.5%) |
| *Citrobacter freundii* | 1 | 1.7 | 2 | 4.3 | 1 | 1.7 | 1 | 2.4 | 5 (2.4%) | 3 (60%) |
| *Citrobacter brachii* | - | - | 1 | 2.1 | 2 | 3.3 | 2 | 4.8 | 5 (2.4%) | - |
| *Streptococcus uberis* | - | - | 2 | 4.3 | 1 | 1.7 | - | - | 3 (1.4%) | - |
| *Staphylococcus xylosus* | 1 | 1.7 | 4 | 8.5 | 2 | 3.3 | - | - | 7 (3.4%) | 1 (14.2%) |
| Total | 59 | 100 | 47 | 100 | 60 | 100 | 41 | 100 | 207 | 92 (44.4%) |

*Vibrio anguillarum* was detected, with a relatively high incidence, as member of the Vibrionaceae (*Vibrio* and *Photobacterium*) family which is considered common marine aquatic bacteria and typical of the fish flora. *Vibrio anguillarum* is the causative agent of vibriosis with lethal hemorrhagic septicaemic disease in different marine, fresh, brackish-water fish, bivalves and crustaceans causing severe economic losses world wide[19]. Some *Vibrio* species, *V. cholerae*, *V. vulnificus* and *V. parahaemolyticus* have been identified as human pathogens[20].

Regarding *A. hydrophila*, which is naturally present in aquatic environments; is the causative agent of motile *Aeromonas septicemia* in fish causing high mortality among fresh and marine-water fish throughout the world. *Aeromonas* spp., has been recognized as potential foodborne pathogens. *Aeromonas* spp., can cause infections in humans, including wound infections, septicaemia, respiratory tract infections and self-limiting diarrhoea, particularly in children[21,22].

Concerning *P. fluorescens* isolated with low incidence in present study, it is an aquaculture pathogen that can infect freshwater and salt-water fish throughout the world causing hemorrhagic septicemia (red skin disease) identical to that seen with *Aeromonas septicemias* and appear to be stress related disease[23]. It has been found to cause gastroenteritis in human after fish and fish products consumption and have emerged as an important factor in the pathogenesis of Crohn's disease[24].

With respect to *C. perfringens* which was isolated with low incidence in this study, it is one of the most widely spread pathogens in the environment and is known as a major cause of food poisoning and foodborne outbreaks characterized by diarrhea and abdominal pain. In Japan, about 30 *C. Perfringens* foodborne outbreaks occur every year[25].

Other bacteria identified in this study such as *E. coli* cause a risk to the consumers and responsible for gastroenteritis and food poisoning. The contamination of food of fish origin with pathogenic *E. coli* probably occurs during handling of fish and during the production process[2]. Various members of the Enterobacteriaceae family can be found in the aquatic environment and consider important histamine-producing bacteria, especially in scombroid fish causing histamine food poisoning. Histamine fish poisoning is common and occurs world wide[12,17]. Most outbreaks of fish food poisoning are associated with consumption of raw or insufficiently cooked fish.

Niven's and modified Niven's agar are presumptive, easy, cheap, reliable and adaptive quantitative assays, making them suitable for the enumeration of cadaverine, putrescine and histamine-producing bacteria from fish and environmental samples[26].

In the present study screening of bacterial isolates by Niven's and modified Niven's agar demonstrated that most of these bacterial isolates were positive for cadaverine, putrescine and histamine production respectively. These data were in accordance with what reported in Egypt previously that cadaverine were the highest followed by putrescine and finally histamine in all examined fish products[27]. Rabie *et al.*[7] reported that cadaverine was the major amine detected in Feseekh samples from Egypt at all sampling stages while histamine was the least. Shakila *et al.*[28] have reported that the proportion of cadaverine and putrescine forming bacteria was quite high in the commercial fish samples of Tuticorin region, India than histamine-forming bacteria which was recorded in low numbers. Also, putrescine was the main biogenic amine formed in ice-stored sea bass (*Dicentrarchus labrax*)[17]. Ozogul *et al.*[29] detected putrescine, cadaverine, 2-phenylethylamine, dopamine, agmatine, tryptamine and serotonin in ice storage wild white grouper. Whereas, histamine, spermine and spermidine were never detected. Zhang *et al.*[30] likewise reported putrescine, cadaverine and tyramine were the main BAs formed in silver carp sausages during the entire processing period. In other studies found negligible amount or no histamine was detected in fresh Indian mackerel[31] and Atlantic mackerel[32].

Biogenic amines are usually formed during bacterial enzyme activity. The types and levels of biogenic amines depend on the presence of certain bacterial species and its count. In this study bacterial isolates that showed positive reaction onto Niven's and modified Niven's agar supplemented with lysine, ornithine and histidine to give biogenic amines (cadaverine, putrescine and histamine, respectively) showed that both *Enterobacter aerogenes* and *E. coli* were the highest isolates that showed positive reaction for biogenic amines followed by *Pseudomonas fluorescens*, *Citrobacter freundii* and *Proteus vulgaris*. Regarding *Micrococcus* spp. isolates showed positive reaction with lower incidence followed by *A. hydrophila* and *Vibrio anguillarum*. On the other hand the lowest bacterial isolates that showed positive reaction were *C. perfringens* and *S. xylosus*. As reported before, *Citrobacter*, *Klebsiella*, *Escherichia*, *Proteus*, *Salmonella* and *Shigella* species of family Enterobacteriaceae are associated with production of considerable amounts of putrescine, cadaverine and histamine in fish and meat products[12,33,34]. These results were nearly similar to that obtained by Kim *et al.*[35] and Tsai *et al.*[36] who found that

*Proteus vulgaris*, *Proteus mirabilis*, *Enterobacter aerogenes* and *Citrobacter freundii* strains possess histidine decarboxylase. While, the weak histamine formers were identified as *V. parahaemolyticus* and *V. alginolyticus*. Also, *Vibrio* sp., *Bacillus* sp., *Pseudomonas* sp., *Staphylococcus* sp., *Enterobacter* sp., *Morganella* sp., and *Klebsiella* sp., were isolated and confirmed by Niven's medium in uneviscerated Indian mackerel (*R. kanagurta*)[37]. Three histamine positive isolates: *Morganella morganii*, *Plesiomonas shigelloides* and *Aeromonas hydrophila* were tested qualitatively for the production of arginine, lysine and tyrosine decarboxylases and also for histamine production in Niven broth[38] and *Vibrio* sp., especially *Vibrio alginolyticus* is one of bacteria that forms histamine[39] .

Regarding the role of *Pseudomonas* sp., in biogenic amines production, the production of putrescine has been confirmed to occur by *Pseudomonas* species. *Pseudomonas* were dominant in all cases for whole, gutted and filleted sea bass over the entire storage period of 16 days[17]. Gram and Huss[40], found that *Pseudomonas* spp., and *Shewanella putrefaciens* are the specific spoilage bacteria of iced fresh fish regardless of the origin of the fish. Additionally, *Pseudomonads*, $H_2S$-producing bacteria and to a lesser extent, Enterobacteriaceae were the dominant microorganisms in both whole and filleted freshwater rainbow trout (*Oncorhynchus mykiss*) stored on ice[41].

Concerning gram-positive bacteria identified in this study, *Micrococcus* sp., were the most BA producing bacteria identified in this study. Micrococcaceae family was found to be the third most abundant group of bacteria isolated from fresh water fish[42]. Also, decarboxylase activity was observed in some species belonging to the genera *Micrococcus* and *Staphylococcus* in fermented sausages[43]. *Staphylococcus* isolates were coagulase negative and were identified as *Staphylococcus xylosus* that were giving positive reaction with ornithine in this study considered as amine-negative starters and delay putrescine and cadaverine formation during the ripening and storage of dry fermented sausages[44]. Other authors reported that *S. epidermidis* and *S. capitis* isolated from salted Spanish anchovies produced more than1,000 and 400 ppm of histamine, respectively[45]. Also, Kung *et al.,*[13] identified *Raoultella ornithinolytica* (one strain), *Enterobacter aerogenes* (one strain) and *Staphylococcus pasteuri* (2 strains) were as histamine formers in Tuna Sausage products. Concerning histamine production by *C. perfringens* in scombrotoxin-forming fish was minimal compared with that by gram-negative bacteria and that *C. perfringens* may not be an important bacterial species associated with scombrotoxin fish poisoning[46].

## CONCLUSION

A wide range of microorganisms were identified in this study which are causal agents of foodborne illnesses and histamine intoxication in humans. Salted fish (fesikh and moloha) contain high amounts of biogenic amines especially cadaverine and putrescine, so the consumers should avoid the cheap and low quality fish products. The Niven's method is considered as a suitable and presumptive method in detection of biogenic amines in environmental samples. Application of early detection of biogenic amines producing bacteria and improvement of hygienic practices could reduce the risk of food poisoning.

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