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DETECTION OF *MORGANELLA MORGANII* IN FROZEN MACKEREL VIA MULTIPLEX PCR RECOGNITION AND ASSIMILATION

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Received: 19 May 2024; **Accepted:** 1 Jun 2024

ABSTRACT

Morganella morganii is a psychrotolerant organism recognized as a histamine-producing bacteria by decarboxylation of histidine amino acid, it has been involved in cases of histamine fish poisoning reported in different seafood products. In this study, we collected a total of 125 random samples of frozen Mackerel fish stored at -18°C from different fish markets in New Valley Governorate, Egypt. Through isolation from the tissue of fish samples on Chromogenic agar plates, bacteria forming light brown colonies were identified. Confirmatory identification of bacteria was conducted using 16S rRNA as a species-specific gene, revealing the presence of 14 *M. morganii* strains from samples, representing an incidence of 11.2% of all samples. *Morganella morganii* is a prominent adaptive nosocomial bacterium that can cause urinary tract infections and bacteremia.

Keywords: *Morganella morganii, Mackerel fish, Histamine fish poisoning, PCR*

INTRODUCTION

Morganella morganii is a Gram-negative, facultative anaerobic bacterium, that lurks in various environments, from freshwater and soil to the digestive tracts of humans and animals. This adaptability underscores its role as an opportunistic nosocomial pathogen, capable of causing a spectrum of infections, from urinary tract infections to systemic bacteremia (Lin *et al.*, 2015). Its presence in diverse habitats, coupled with its remarkable virulence and resistance traits make *M. morganii* a formidable foe in clinical settings (Liu *et al.*, 2016).

Belonging to the Proteeae tribe within the Enterobacteriaceae family, *M. morganii* is distinguished by its ability to produce extended-spectrum beta-lactamases (ESBLs) and other resistance mechanisms, challenging even the most robust antibiotic treatments (Singla *et al.*, 2010). This bacterium is not just a clinical nuisance; it is a severe threat. It can cause devastating conditions like endophthalmitis, central nervous system infections, postoperative wound infections, and bacteremia. In particular, infections related to urinary and hepatobiliary tract disturbances are often

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fatal, demanding urgent and effective medical interventions (Chen *et al.,* 2012; Behera *et al.,* 2023).

However, the menace of *M. morganii* extends beyond hospital walls. Fish, with their high histidine content, are prime targets for histamine production by this bacterium. Through the decarboxylation of histidine, *M. morganii* produces histamine, leading to scombroid poisoning. Victims suffer from symptoms such as rash, nausea, vomiting, diarrhea, and headaches. To prevent such incidents, the European Commission has mandated that the amount of histamine in fish should not exceed 200 mg/kg (Oktariani *et al.,* 2022). Rigorous detection and control measures, employing methods like high-performance liquid chromatography (HPLC) and enzyme-linked immunosorbent assay (ELISA), are critical to mitigating these risks (Lehane and Olley, 2000; Bolon *et al.,* 2023).

In this context, our study takes on a vital mission. We aim to assess the microbial quality and safety of frozen mackerel fish from the New Valley Governorate in Egypt. By using advanced molecular techniques such as polymerase chain reaction (PCR), we intend to pinpoint the prevalence of *M. morganii*. This endeavor will not only shed light on the potential hazards of histamine fish poisoning but also pave the way for improved seafood safety protocols. Through this research, we aspire to enhance public health defenses against this insidious pathogen, ensuring safer consumption of seafood and a deeper understanding of *M. morganii's* impacts.

MATERIALS AND METHODS

1. Samples collection

A total of 125 random samples of Mackerel fish frozen at -18 ºC were purchased at local department stores and markets in New Valley Governorate from 2022 to 2023, each sample was gained in its casing as it was sold to consumers then these samples were transported in an ice box to the laboratory and analyze.

2. Preparation of samples (Elbarbary *et al.,* 2023).

In the laboratory, frozen samples were defrosted by overnight refrigeration. (3° C±0.5); every sample was thoroughly and carefully removed from its packaging and subjected to the following examination

Under investigation, ten grams of each sample of fish flesh were aseptically weighed and homogenized for two min. using Stomacher 400 Circulator Lab Blender in a very sterile bag containing 90 ml of sterile 0.1% peptone water.

3. Isolation of *Morganella morganii* (Gopinathan *et al.,* 2021).

Enrichment procedures

One ml of the prepared sample was added to 10 ml of tryptic soy broth (Millipore 146317), mixed using vortex then incubated at 37 ºC for 24hr.

Selective plating

Chromogenic (HiCrome UTI) agar plate (HiMEDIA M1353) was streaked by a loop full from incubated Tryptic soy broth and then incubated in an inverted position at 37 ºC for 24 hr. A separate colony was picked up and streaked onto tryptic soy agar (Millipore105458) slope for further identification and then incubated for 24 hr at 37 ºC.

4. Identification of *Morganella morganii* according to (Zaric *et al.,* 2021)

Traditional Biochemical Tests: All strains of bacteria were morphologically evaluated using Gram's stain and submitted to certain biochemical tests, including: motility test, IMViC tests, oxidase test, catalase test, urease test, gas and H2S production, gelatinase test.

5. Detection of *Morganella morganii* by Polymerase Chain Reaction (PCR) (Holasoo *et al.*, 2022)

PCR was used to demonstrate 16S rRNA as a species-specific gene for confirmation identification of *Morganella morganii*, as shown below:

The amplification was done using a Thermal Cycler (Master Cycler, Eppendorf, Hamburg, Germany). Therefore, PCR mixture contained 12.5 µl of 2X Master Mix (Amplicon, Odense, Denmark), 0.2 pmol of primer (100 pmol/µl), 1 µl of template DNA (100 ng), and 9.5 µl of distilled water in the final amount of 25 µl.

Accurately, the PCR cycling procedure was as follows: an initial denaturation at 95 °C for 3 minutes, followed by 34 cycles of denaturation at 95 °C for 30 minutes, annealing at 58 °C for 30 minutes, extension at 72 °C for 90 seconds, and a final extension at 72 °C for 7 minutes.

Finally, 5 µl of each amplicon was electrophoresed in 1.2% agarose gel for one hour at 90 V. The agarose gel was stained with ethidium bromide (0.5µg/ml), collected, and visualized on a UV transilluminator before photographing. The fragment sizes were determined using a 100-bp-plus DNA ladder.

Table 1: Primer sequences of *M. morganii* used for PCR system.

Target gene	Oligonucleotide sequence (5′ → 3′)	Product size (bp)	References
16S rRNA (27F)	**5′** AAGAGTTTGATCCTGGCTCAG **′3**	1500	**Wang *et al.* (2017)**
16S rRNA (1492R)	**5′** GGTTACCTTGTTACGACTT **′3**		

RESULTS

The colony structure of suspected *M. Morganii* strains on chromogenic agar appears light brown colonies also it is a Gram-negative rod in a smear investigated under the light microscope and the results of the biochemical test showed that the bacterium is motile, oxidase negative and catalase positive. They are facultative anaerobes that produce acid and gas through D-glucose metabolism; they are indole positive, VP negative, MR positive, and urease positive.

Table 2: Incidence of *Morganella morganii* isolated from the examined samples of frozen Mackerel fish.

Mackerel fish	No. of examined samples	*Morganella morganii*	
		No	%
	125	14	11.2

Table (2) shows that fourteen bacterial isolates were obtained from 125 frozen Mackerel fish samples with an incidence of 11.2%. The suspected *M. moganii* was isolated according to colony morphology on chromogenic agar.

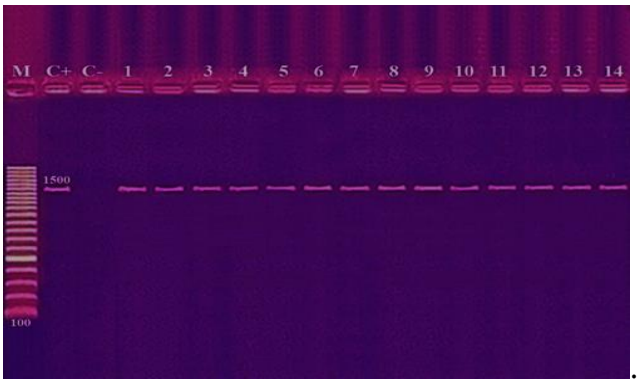

Fig. (1): Agarose gel electrophoresis of PCR of *16S rRNA* (1500 bp) as species specific gene for detection of *Morganella morganii*.

In figure (1) **Lanes from 1 to 14** show Positive for *16S rRNA* of *Morganella morganii*.
Lane M: 100 bp ladder as molecular size DNA marker.
Lane C+: Control positive strain for *16S rRNA.*
Lane C-: Control negative.

DISCUSSION

The microorganisms in fish appropriate for human intake are influenced by the environmental conditions in their native environment. Fish from temperate waters, like Mackerel, are often home to psychrotolerant Gram-negative bacteria (Visciano *et al.,* 2012).

Release of histidine decarboxylase, the enzyme responsible for conversion of histidine to histamine by microorganisms, might occur in the early stages of fish handling or due to fluctuation of temperature during storage (Tahmouzi *et al.,* 2013). In one experiment, Staruszkiewicz *et al.* (2004) observed that histidine decarboxylase activity was retained in some frozen fish samples and could result in further increases in histamine production during the thawing process. Defrosting the frozen is considered a crucial phase as the psychrotolerant bacteria produce toxic concentrations of histamine at 0–5 ^{o}C and appear to be important in histamine formation in chilled fish (Emborg *et al.,* 2006).

Histamine-producing bacteria (HPB), including *M. morganii,* can invade the edible portions of fish at any point from harvest to processing, distribution, and even after cooking. They naturally reside in the gut, gills, and skin of the fish, ready to strike at the slightest mishandling (Bjornsdottir-Butler *et al.,* 2015).

In the vibrant realm of seafood markets, frozen Mackerel fish await their destiny amidst bustling activity. Unbeknownst to many consumers, these seemingly innocent fish conceal a subtle intruder: *Morganella morganii*. Our meticulous examination unveiled the presence of this bacterium in 11.2% of the Mackerel samples after being thawed at a chilled temperature 3◦C±1. Meanwhile, Mohamed *et al.* (2017) uncovered a more pronounced prevalence, with *Morganella morganii* dominating at 18.52% from a staggering 83 isolates across all analyzed fish specimens. Ali *et al.* (2021) reported an 8% incidence in Tilapia, showing variability in incidence rates across different types of fish.

Temperature plays a critical role in the life of *M. morganii*. Higher temperatures boost its growth and metabolic activity, leading to increased histamine production. However, freezing and refrigeration slow down these processes but do not completely halt them. Our findings are supported by studies showing that temperature abuse storing fish at temperatures above 15°C significantly

enhances bacterial multiplication and histidine decarboxylase activity (Lehane and Olley, 2000).

Elsenduony *et al.* (2016) show results higher than our results he revealed that the most common isolated species from frozen fish stored at -18 ºC were *Morganella morganii* and the obtained results were 41 (39.8 %) from a total number of 120 examined fish samples, examined samples of frozen mackerel, frozen sardine, frozen herring and frozen tuna revealed results 24 (68.5%), 20 (57,1), 28 (80%),15(100%), respectively. also it was isolated from frozen meat 8(25%) and frozen sausage 5(16.1%) both were stored at -18 ºC.

Besides, Elsenduony *et al.,* (2016) said that the most abundant bacteria is *Morganella morganii*, which has been reported as the most efficient decarboxylating microorganism in different studies and plays the major role in histamine formation in fish that is improperly handled.

Marg, (2012) revealed that psychrotolerant, mesophilic and other enteric bacteria such as *Morganella* sp., were isolated from both eviscerated and uneviscerated Indian mackerel stored at -20 ºC. Further, their histamine-producing ability was confirmed by TLC analysis

Kim *et al.,* (2002) Viewed that they could isolate *M. morganii* from mackerel fish stored at -30º C with counts of detected *M. morganii* were 1.4 $x10^5$ CFU/g after 2 months storage and 2.1x 10^4 CFU/g after 3 months storage, while when mackerel fish stored at -20º C counts of *M. morganii* were 1.0 $x10^5$ CFU/g in 2 months storage and 1.6$x10^4$ CFU/g in 3 months storage.

Ben-Gigirey *et al.* (2000) observed that *M. morganii* could not be isolated from albacore fish stored at 0°C, but was present at higher temperatures (25°C and 30°C). This aligns with our observation that temperature abuse enhances bacterial growth and histamine production.

M. morganii is notorious for its role in scombroid poisoning. This cunning bacterium thrives under favorable conditions, producing large amounts of histamine. Histamine, a simple compound, can become a potent toxin when accumulated in food. *M. morganii's* ability to decarboxylate amino acids, especially histidine, makes it a formidable enemy in the world of food safety. It is frequently found in fish linked to scombroid food poisoning (SFP) and is known for its high histamine production (Economou *et al.*, 2007).

Our results support the findings by Economou *et al.* (2007) and Kim *et al.* (2001), who identified *M. morganii* as a prolific histamine producer. This is critical since histamine remains stable even after cooking, posing a significant health risk (Lorca *et al.*, 2001).

Kim *et al.* (2001) noted that *M. morganii* was the most prolific and prevalent HPB isolated from fresh Pacific Mackerel. Our study echoes this finding, confirming the bacterium's significant presence and histamine-producing capability in Mackerel. The precision of our PCR assays in detecting *M. morganii* underscores the importance of molecular techniques in food safety.

Histamine's heat stability poses a unique challenge. Once formed, histamine survives cooking and smoking, making raw fish with elevated histamine levels a hidden hazard (Lorca *et al.,* 2001). This emphasizes the need for stringent control measures during all stages of fish handling to prevent histamine formation.

Cross-contamination in seafood processing environments remains a significant concern. *M. morganii* can cling to the slime layer of fish, spreading through contact with contaminated surfaces during handling and transport. Our findings corroborate these

concerns, highlighting the role of contaminated surfaces and ice in spreading *M. morganii*. Processing stages can further disseminate the bacteria from the gastrointestinal tract and gills to muscle surfaces (Lorca *et al.*, 2001). The extensive use of ice in preserving seafood complicates this issue, as contaminated ice and meltwater can facilitate bacterial spread. Fernandes (2009) suggested using ice prepared with safe sanitizers could mitigate this risk.

The seafood industry faces a daunting challenge in controlling histamine formation. A misunderstanding of critical control points for contamination by abundant histamine producers such as *M. morganii* has delayed the implementation of effective Hazard Analysis Critical Control Point (HACCP) systems. Monitoring the presence and proliferation of *M. morganii* is essential for implementing HACCP protocols to control histamine levels in fish products (Kim *et al.*, 2002).

CONCLUSION

In conclusion, the study successfully isolated and identified *Morganella morganii* from frozen Mackerel fish samples, highlighting its prevalence and potential health risks. The combination of morphological, biochemical, and molecular methods provided a comprehensive approach to bacterial identification. These results emphasize the importance of continuous monitoring and improving food safety practices to protect public health.

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الكشف عن مورجانيلا مورجاني في سمك الماكريل المجمد من خلال تعرف واستيعاب PCR المتعدد

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مورجانيلا مورجاني بكتيريا محبة للبرودة منتجة للهستامين عن طريق نزع الكربوكسيل من الحمض الأميني الهستيدين، وقد تورطت في حالات تسمم الأسماك بالهستامين الموجودة في العديد من منتجات المأكولات البحرية, وهدفت هذه الدراسة الى الكشف عن بكتيريا المورجانيلا مورجاني في اسماك الماكريل المجمدة وذلك لتقييم جودتها الصحية حيث قمنا بجمع إجمالي ١٢٥ عينة عشوائية من أسماك الماكريل المجمدة المخزنة في درجة حرارة -١٨ درجة مئوية من أسواق الأسماك المختلفة في محافظة الوادي الجديد، مصرومن خلال العزل من أنسجة عينات الأسماك على أطباق آجار كروموجينيك، تم التعرف على البكتيريا التي تشكل مستعمرات بنية فاتحة. تم إجراء تحديد مؤكد للبكتيريا باستخدام 16S rRNA باعتباره جينًا خاصًا بالأنواع، مما كشف عن وجود ١٤ سلالة من مورجانيلا مورجاني من العينات، وهو ما يمثل نسبة حدوث ١١,٢% من جميع العينات. تعد المورجانيلا المورجانية من العوامل المسببة للأمراض الانتهازية القادرة على التسبب في التهابات المسالك البولية وتجرثم الدم و خلص البحث إلى أن هذه النتائج تؤكد على أهمية المراقبة المستمرة وتحسين ممارسات سلامة الأغذية لحماية الصحة العامة لتجنب العدوي ببكتيريا المورجانيلا مورجانى وذلك لانها من الممكن ان تنتشر من خلال ملامسة الأسطح الملوثة أثناء المناولة والنقل.