FOODBORNE PATHOGENS AND DISEASE
Volume 16, Number 9, 2019

DOI: 10.1089/fpd.2018.2616

Histamine-Producing Bacteria and Histamine Induction in Retail Sardine and Mackerel from Fish Markets in Egypt

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Abstract

This study examined the occurrence of histamine-producing bacteria (HPB) and histamine induction in retail sardine and mackerel in Egypt; and whether the fish vendors play a role in the transmission of HPB. Fish were collected from the fish markets, additionally; hand swab samples were taken from the fish vendors. All samples were cultured on modified Niven's medium (MNM); the positive colonies were subcultured on Violet Red Bile Glucose (VRBG) agar, followed by biochemical identification and histidine decarboxylase (*hdc*)-gene-PCR of the VRBG-positive isolates. The *hdc*-gene-positive fish and human isolates were subjected to partial *hdc*-gene-sequencing and phylogenetic analysis. Production of histamine in the fish muscles was measured by high-performance liquid chromatography. A higher percentage of sardine showed the presence of MNM-positive bacteria (84%) than mackerel (53%). *Enterobacteriaceae* was the dominant family; the most frequent species were *Enterobacter cloacae*, *Raoultella planticola, Citrobacter freundii*, and *Enterobacter aerogenes*. Higher proportion of the *R. planticola* isolates were *hdc* positive as compared with the other species. Only 32% sardine and 17% mackerel of the MNM-positive isolates carried the *hdc* gene. Fish muscles that contain *hdc*-positive bacteria exhibit higher levels of histamine (median 86; IQR 80–1112 mg/kg) than those with *hdc*-negative bacteria (48; 75–223 mg/kg). The level of histamine was significantly higher in sardine (109; 104–1094 mg/kg) than in mackerel (40; 49–106 mg/kg). The 20 fish vendor samples were MNM positive, 2 of them were *hdc*-gene positive. The close genetic relatedness between the human and fish strains isolated from the same markets suggests a possible bidirectional transmission of the HPB. This warns for the presence of HPB carrying *hdc* gene in retail sardine and mackerel, which is associated with a relatively high level of histamine. Regular inspection of the fish markets is required, including accurate determination of HPB by using a combination of the MNM culture, *hdc*-gene PCR, and measurement of histamine level.

Keywords: histamine fish poisoning, histamine-producing bacteria, *Enterobacteriaceae*, *Raoultella planticola*, retail market fish, sardine, mackerel, *hdc* gene, Egypt

Introduction

Fish is a low-calorie high-protein food; its consumption might result in some foodborne illnesses and outbreaks (Iwamoto *et al.*, 2010). Of particular interest is histamine fish poisoning (HFP), which is caused by eating spoiled fish that contain high levels of histamine (Taylor *et al.*, 1989; Visciano *et al.*, 2012; Feng *et al.*, 2016).

Scombroid and other dark muscle fish such as tuna, bonito, mackerel, blue fish, dolphin, sardine, carangids, herring, and anchovies are prone to form histamine, as their muscles are histdine-rich. (Choudhury *et al.*, 2008; Feng *et al.*, 2016). Histamine formation results from histdine decarboxylation through histamine-producing bacteria (HPB) containing histidine decarboxylase (HDC) enzyme. (Lerke *et al.*, 1978; Ferrario *et al.*, 2012; Wongsariya *et al.*, 2016).

This process can start as soon as the fish dies and kept at temperature above 4°C for an extended period (FAO/WHO, 2012). Once the HDC enzyme is synthesized, it continues to produce histamine even if the bacteria get inactivated (EFSA, 2011; FDA, 2011). Cooking can inhibit the HPB and the HDC, while histamine cannot be degraded by cooking, smoking, or canning of fish, indicating that both raw and cooked fish might cause HFP (Hungerfood, 2010). Accordingly, the legal level of histamine should not exceed 200 mg/kg in marketed fish species rich in histidine (EFSA, 2011; FAO/WHO, 2012, 2018). The major HPB reported in fish are the family *Enterobacteriaceae* (Kim *et al.*, 2003; Klanian *et al.*, 2018). Of these, *Morganella morganii,*

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Enterobacter aerogenes, Enterobacter cloacae, Raoultella planticola, Raoultella ornithinolytica, and *Photobacterium damselae* are particularly high histamine producers. However, other species including *Citrobacter freundi, Vibrio alginolyticus*, and *Escherichia coli* are weak histamine producers (Takahashi *et al.,* 2003).

So far, little information is available about HPB in Egypt. Therefore, the objectives of this study were to investigate the presence of HPB and histamine production in retail sardine and mackerel, two types of commonly consumed histidine-rich fish in Egypt; and to examine whether the fish vendors play a role in the transmission of the HPB. Accordingly, we collected muscle samples from the fish at the markets, as well as hand swab samples from the fish vendors. All samples were subjected to bacterial isolation and identification of HP-*Enterobacteriaceae*, followed by molecular detection of *hdc* gene among the isolates. The level of histamine in the fish muscles was measured by high-performance liquid chromatography (HPLC). The genetic relatedness of the fish and human isolates collected from the same markets was determined by partial sequencing of the *hdc* gene.

Materials and Methods

Samples

The study included apparently healthy frozen imported mackerel ($n=100$) and fresh local sardine ($n=57$); whole fish were taken and placed in sterile polyethylene bags. Hand swab samples were collected from the fish vendors ($n=20$); none of them had allergic symptoms. One swab per individual was used, rubbed in the interdigital spaces, nails, palms, and on the back of the hands, then inoculated into sterile tubes containing 9 mL of 0.1% peptone water solution. Both fish and human samples were transported to the laboratory on ice. All samples were collected from randomly selected fish markets located in EL-Giza and Alexandria Governorates, during the period from March 2016 to April 2017. Protocols for collection of samples as well as all methods were performed in accordance with the guidelines and regulations of Cairo University Council, Faculty of Veterinary Medicine, Egypt. Written consent to use samples was obtained from each person.

Bacterial isolation and identification

Ten gram muscles were taken from the dorsal, abdominal, and tail parts of each fish. The muscles were inoculated into 9 mL of 0.1% peptone water, and homogenized using Stomacher® 400 blender (Seward lab, Worthing, United Kingdom) (Fletcher *et al.*, 1998).

Isolation of HPB. The modified Niven's medium (MNM) was used for initial selection of HPB. One milliliter from each of the fish homogenates (mackerel, $n=100$; sardine, $n=57$) as well as the human hand swab suspensions ($n=20$) were streaked separately into the MNM prepared according to Niven *et al.* (1981); Joosten and Northolt (1989); and Mavromatis and Quantick (2002). The plates were incubated at 37°C for 48–72 h and were examined every 24 h. The HPB colonies are purple in color surrounded by purple halo on a yellowish background.

Isolation of histamine-producing *Enterobacteriaceae*. The presumptive MNM-positive isolates (mackerel, $n=58$; sardine, $n=44$; humans, $n=20$) were streaked onto plates of Violet Red Bile Glucose (VRBG; LAB M, Heywood, United Kingdom) agar, a selective medium for isolation of *Enterobacteriaceae*, and were incubated at 37°C for 24 h (Mossel, 1985). The suspected colonies were purified through subculture on Tryptic Soya Agar (LAB M) plates, and were examined for morphological and phenotypic traits. Typical colonies of *Enterobacteriaceae* are Gram negative, round, purple-pink surrounded by purple halo.

c. Biochemical identification. The VRBG colonies subcultured on Tryptic Soya Agar were subjected to the traditional oxidase biochemical test according to Shore and Isenberg (2007), and confirmed with API 20E kit (BioMérieux, Marcy-l'Étoile, France). API 20NE kit was used to confirm the identity of the non-*Enterobacteriaceae* strains that grew on the VRBG. The accuracy of the kit results was 100% as determined by API web software (BioMérieux).

*III. Molecular detection of histidine decarboxylase (*hdc*) gene*

All the MNM-VRBG-positive isolates (mackerel, $n=58$; sardine, $n=44$; humans, $n=20$) were examined for the presence of the *hdc* gene. Genomic DNA was extracted using the rapid boiling method (Reischl *et al.*, 1994) and subjected to conventional PCR using specific oligonucleotide primers: forward 5′-TGGGGTTATGTSACCAATGG-3′, reverse 5′-GTRTGGCCGTTACGY GARCC-3′ with an amplicon size of 571 bp (Wongsariya *et al.*, 2016). PCR mixtures for a total volume of 25 μL consisted of 3 μL of template DNA from each isolate, 12.5 μL of GT-PCR master mix (Takara Bio, Inc., Shiga, Japan), 1 μL of 10 pmol of each primer, and 8.5 μL nuclease-free water. All PCR mixtures were performed in duplicates and subjected to 35 cycles of 94°C for 1 min, 54°C for 1 min, and 72°C for 4 min. The PCR products were electrophoresed on 1.5% agarose gel, a DNA marker (Gene Ruler™ 100 bp DNA Ladder, Vivantis Technologies Sdn. Bhd., Selangor Darul Ehsan, Malaysia) was run simultaneously.

Sequencing of the PCR-amplified hdc*-gene fragment*

The amplified *hdc*-gene PCR product obtained from the fish ($n=6$) and human ($n=2$) samples collected from the same markets was purified using Qiaquick PCR Product extraction kit (Qiagen, Hombrechtikon, Switzerland). The purified PCR products were sequenced using BigDye Terminator V3.1 sequencing kit (Applied Biosystems, Waltham, MA; the obtained nucleotide sequences were deposited in the GenBank.

Phylogenetic analysis. The obtained nucleotide sequences were compared with those available in public domains using the NCBI-BLAST server, and were imported into the BioEdit program version 7.0.1.4 for multiple alignments using the BioEdit Clustal W program. Phylogenetic analysis was performed with the MEGA program version 7 using the neighbor-joining approach.

Measurement of levels of histamine in fish muscles using HPLC

Fish samples that showed the presence of MNM-VRBG-positive bacteria (mackerel, $n=49$; sardine, $n=39$) were subjected to HPLC. Preparation of samples was performed according to Frattini and Lionetti (1998). In brief, 5 g muscle from each sample was homogenized separately using 10 mL trichloroacetic acid extracting solution (Thermo Fischer Scientific, Waltham, MA) and centrifugation. The supernatant was filtered, mixed with 1 mL of 1 M NaOH (Thermo Fischer Scientific), and incubated at RT for 5 min. One milliliter of o-phthalaldehyde (Acros Organics, Geel, Belgium) and 3 mL ethyl acetate (Thermo Fischer Scientific) were then added, and the homogenate was precipitated by centrifugation. The pellets were dried on a rotatory evaporator, then resuspended in 1 ml acetonitrile (Thermo Fischer Scientific). A histamine dihydrochloride standard solution (Sigma-Aldrich, St. Louis, MO) was serially diluted in 0.1 M HCl (Thermo Fischer Scientific) to obtain the following concentrations: 0.5, 1, 2, 4, 8, 16, 32, and 64 mg/L. The standard and the processed samples were injected into intersil C18 columns (4.6×250 mm, size particle 5 μm; Agilent Technologies, Waldbronn, Germany); each determination was injected twice. The chromatographic separation was performed using 1050 HPLC (Agilent Technologies) according to Tahmouzi *et al.* (2011).

Calculation of histamine concentration. The histamine content in mg/kg fish was calculated using the following formula according to Jinadasa *et al.* (2016): [The level of histamine in the extract (mg/L) as extrapolated from the standard curve/sample weight (5 g)] $\times$ 10 (dilution factor).

Statistical analysis

Statistical analysis was done using NCSS 9 software program (NCSS, Kaysville). Two proportion Z test was used to compare the occurrence of HPB between mackerel and sardine. The same test was used to compare expression of *hdc* gene between HPB present in mackerel and those in sardine. Since the data of the histamine levels were not normally distributed, the Kruskal–Wallis test was used to analyze the difference in the level of histamine between sardine and mackerel as well as between *hdc*-gene-positive and *hdc*-gene-negative groups. On each occasion, significance level was observed at 0.05 ($p\leq0.05$).

Results

Occurrence of HPB carrying hdc *gene in retail mackerel and sardine fish*

Among the 100 examined mackerel samples, 53 (53%) were positive in MNM, a selective medium for selection of HPB (Table 1). Compared with mackerel, a significantly higher percentage (84%, 48/57) of sardine were positive in MNM. The majority of the MNM isolates grew on the *Enterobacteriaceae*-selective medium VRBG, as found in the mackerel (49 of the 53 MNM) and sardine (39 of the 48 MNM) strains. Biochemical identification confirmed that almost all of the VRBG-positive isolates are *Enterobacteriaceae* except for trivial numbers that were non-*Enterobacteriaceae* (Table 1). A variety of bacterial species were identified among the mackerel ($n=58$) and sardine ($n=44$) MNM-VRBG-positive isolates, the most common were *E. cloacae*, *R. planticola, Citrobacter freundii*, *and E. aerogenes* (Table 2). All the identified isolates were subjected to *hdc*-gene PCR; of the 58 mackerel MNM-VRBG-positive isolates, 9 (17%) carried the *hdc* gene; and among the 44 sardine MNM-VRBG-positive isolates, 12 (32%) harbored the *hdc* gene (Table 2). The proportion of *hdc*-positive strains were highest among *R. planticola* (7/15) than *E. aerogenes* (4/12), *C. freundii* (2/14), and *E. cloacae* (2/16).

Significantly higher level of histamine in the muscles of sardine than in mackerel

The production of histamine in the fish muscles that showed the presence of MNM-VRBG-positive bacteria was measured by HPLC (Fig. 1). Interestingly, there was an overall significantly higher level of histamine in the muscles of sardine (median 109; IQR 104–1094 mg/kg) than that observed in mackerel (40; 49–106 mg/kg). In addition, sardine containing *hdc*-gene-negative bacteria had higher level of histamine than mackerel with *hdc*-gene-negative bacteria. This was also the case between sardine containing *hdc*-gene-positive bacteria and mackerel with *hdc*-gene-positive bacteria. However, the level of histamine was higher in fish that contain *hdc*-positive bacteria (86; 80–1112 mg/kg) than in fish with *hdc*-negative bacteria (48; 57–223 mg/kg); the difference was not statistically significant ($p=0.37$).

Finding of HPB in hand swabs from the fish vendors

Hand swabs were taken from humans working in contact with the fish at the same markets. All the 20 examined samples

Table 1. Prevalence of Histamine-Producing Bacteria in Fish Based on Culture on Modified Niven's Medium, Followed by Culture on Violet Red Bile Glucose Agar

		Number of MNM-positive isolates		
			Number of VRBG-positive samples	
Species of fish	*Number of tested samples*	*Total No (%)*	*Enterobacteriaceae*	*non-Enterobacteriaceae*
Mackerel	100	53 (53%)*s	45	4
Sardine	57	48 (84%)	38	1
Total	157	101 (64%)	83	5

*s denotes significant difference between mackerel and sardine using two proportion Z statistical test, $p=0.0002$.
MNM, modified Niven's medium; VRBG, Violet Red Bile Glucose.

Table 2. Identification of the Species of HPB-VRBG-Positive Strains Isolated from Fish as Performed by API20E and Number of Isolates That Carry Histidine Decarboxylase Gene(*hdc*) as Detected by PCR

	Number of HPB-VRBG-positive isolates			*Number of hdc-positive isolates*		
Species of HPB-VRBG-positive strains	*Mackerel*	*Sardine*	*Total*	*Mackerel*	*Sardine*	*Total*
Enterobacter cloacae	10	6	16	2	0	2
Raoultella planticola	4	11	15	2	5	7
Citrobacter freundii	7	7	14	0	2	2
Enterobacter aerogenes	9	3	12	3	1	4
Serratia rubidaea	3	1	4	0	1	1
Klebsiella pneumoniae	2	2	4	0	0	0
Escherichia coli	2	2	4	0	0	0
Proteus mirabilis	2	1	3	1	0	1
Enterobacter sakazakii	2	1	3	0	0	0
Citrobacter amalonaticus	2	1	3	0	0	0
Erwinia spp.	0	2	2	0	2	2
Morganella morganii	1	1	2	1	1	2
Citrobacter braakii	2	0	2	1	0	1
Serratia liquefaciens	1	1	2	0	1	1
Acinetobacter baumannii	2	0	2	0	0	0
Citrobacter youngae	1	0	1	0	1	1
Serratia odorifera	1	0	1	0	0	0
Hafnia alvei	0	1	1	0	0	0
Acinetobacter calcoaceticus	1	0	1	0	0	0
Enterobacter amnigenus	0	1	1	0	0	0
Enterobacter asburiae	0	1	1	0	0	0
Enterobacter cancerogens	1	0	1	0	0	0
Escherichia fergusonii	0	1	1	0	0	0
Salmonella gallinerum	1	0	1	0	0	0
Salmonella choleraesuis	1	0	1	0	0	0
Yersinia enterocolitica	1	0	1	0	0	0
Aeromonas hydrophila	1	0	1	0	0	0
Pseudomonas luteala	0	1	1	0	0	0
Moellerella wisconsensis	1	0	1	0	0	0
Total number	58	44	102	10 (17%)[ns]	14 (32%)	24 (23.5%)

[ns]Points to nonsignificant difference between mackerel and sardine as determined by two proportional Z statistical test, $p = 0.0856$.
HPB, histamine-producing bacteria.

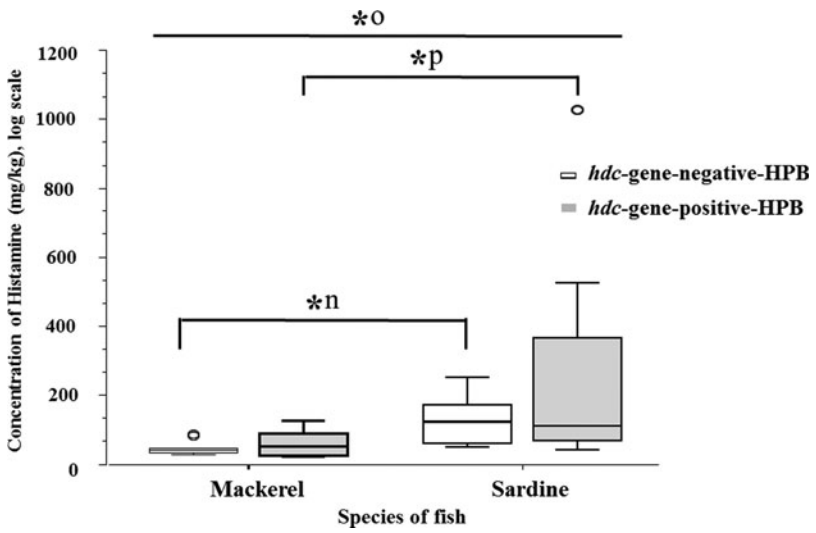

FIG. 1. Comparing the concentration of histamine between mackerel and sardine in regard to the presence of HPB with or without *hdc* gene. The histamine level was measured in the fish muscles by using HPLC and was expressed as mg/kg. This was then compared between the species of fish (mackerel and sardine) as overall and in respect to the presence of *hdc*-negative HPB or *hdc*-positive HPB using Kruskal–Wallis (ANOVA). The results are presented as box plots, and the outliers appear in the form of gray circles. *n indicates significant difference between the *hdc*-negative HPB groups from mackerel and those from sardine. *p points to significant difference between the *hdc*-positive HPB from mackerel and those from sardine. *o indicates an overall significant difference between mackerel and sardine. HPB, histamine-producing bacteria; HPLC, high-performance liquid chromatography.

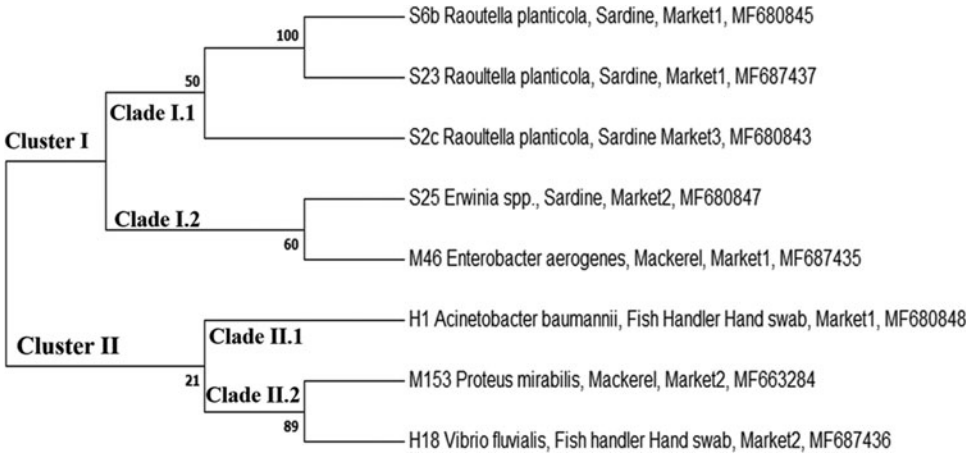

FIG. 2. Identity and evolutionary relation between the HPB strains isolated from fish and humans based on sequencing of the *hdc* gene. Eight bacterial isolates from fish and hand swabs from the fish handlers were subjected to sequencing of histidine decarboxylase (*hdc*) gene and analysis using MEGA7. The results are presented in the form of Bootstrap consensus tree that describes the species of the bacteria and source of the isolates, as well as the accession numbers of the sequences deposited in the NCBI GenBank.

were positive in MNM and in VRBG media (Table 3). Different species of bacteria were detected, including *Klebsiella pneumonia* ($n=4$), *C. freundii* ($n=4$), *E. cloacae* ($n=3$), *Raoultella ornithinolytica* ($n=2$), *E. aerogenes* ($n=2$), *Serratia odorifera* ($n=2$), *Acinetobacter baumannii* ($n=1$), *Vibrio fluvialis* ($n=1$), and *Salmonella cholerasuis* ($n=1$). The *hdc* gene was carried only by the *A. baumannii* and *V. fluvialis* isolates. The *V. fluvialis* isolate was confirmed by sequencing based on Vibrio-genus-specific 16s rRNA (Zheng *et al.*, 2017).

Genetic relatedness of the hdc *gene among the fish and human isolates*

The phylogenetic analysis of the *hdc* gene carried by the isolates collected from the same markets revealed clustering of the isolates into two main clusters based on their evolutionary relationship (Fig. 2). The first main cluster (Cluster I) includes two clades: clade I.1 with the *R. planticola* sardine strains and clade I.2 with the enteric strains (*E. aerogenes* mackerel and *Erwinia* spp. sardine). The second main cluster (Cluster II) also comprises two clades: clade II.1 that contains only the human *Acinetobacter baummanii* strain, while clade II.2 includes the *V. fluvialis* human strain and the *Proteus mirabilis* mackerel strain that showed ~90% similarity.

Table 3. Species of Histamine-Producing Bacteria Isolated from Hand Swabs of Fish Handlers

Species of HPB	*Positive number* (n = *20*)	*hdc gene*
Klebsiella pneumoniae	4	Negative
Citrobacter freundii	4	Negative
Enterobacter cloacae	3	Negative
Klebsiella oxytoca	2	Negative
Enterobacter aerogenes	2	Negative
Serratia odorifera	2	Negative
Acinetobacter baumannii	1	Positive
Vibrio fluvialis	1	Positive
Salmonella choleraesuis	1	Negative

Discussion

In this study, we examined the occurrence of HPB-*Enterobacteriaceae* in retail mackerel and sardine, two types of histidine-rich fish commonly consumed in Egypt, and whether the fish vendors play a role in the transmission of these bacteria. Our results demonstrated that a significantly higher percentage of sardine (84%) samples showed the presence of HPB as compared with mackerel (53%). The majority of the HPB found in sardine (79%) and mackerel (85%) were *Enterobacteriaceae*, which is similar to other studies that reported a proportion of 83%, 93%, and 89% (López-Sabater et al., 1996; Kim *et al.*, 2003; Tembhurne *et al.*, 2013), respectively, indicating a significant role of *Enterobacteriaceae* in histamine production in fish (Koohdar *et al.*, 2011; Klanian *et al.*, 2018). Moreover, we observed the growth of a minor number of non-*Enterobacteriaceae* Gram-negative strains on the VRBG, including *A. baumannii*, *A. calcoaceticus*, *Aeromonas hydrophila*, and *Pseudomonas luteola*, which were further confirmed by API 20NE. In this regard, Eden and Arbon (2014) reported previously that, however, VRBG can allow the growth of some non-*Enterobacteriaceae*; it gives a better recovery of *Enterobacteriaceae* than other media such as MacConkey. The most frequent species of *Enterobacteriaceae* identified among the present mackerel and sardine isolates were *E. cloacae*, *R. planticola*, *C. freundii*, and *E. aerogenes,* which have been implicated in HFP in humans (Kanki *et al.*, 2002; Hu *et al.*, 2012).

Of particular interest is the higher proportion of *hdc*-gene-positive strains among *R. planticola* compared with the other bacterial species, as the role of *R. planticola* as HPB was underestimated for long time (Kanki *et al.*, 2002; Alves *et al.*, 2007; Puerta-Fernandez *et al.*, 2013; Lam and Salit, 2014). In addition, recent studies signified the importance of *R. planticola* as a zoonotic agent (Ershadi *et al.*, 2014; Westerveld *et al.*, 2017). Furthermore, the *hdc*-gene PCR revealed that only 23.5% of the total MNM-VRBG-positive fish isolates carried the *hdc* gene, suggesting that the MNM is highly sensitive and might give false-positive results. Björnsdóttir *et al.* (2010) previously found that the MNM detects both

low- and high HPB, while PCR fails to detect the presence of *hdc* gene in low HPB. This is in agreement with our findings that the histamine level was lower in muscles of fish that contain *hdc*-gene-negative bacteria (median 48; IQR 75–223 mg/kg) than in fish with *hdc*-gene-positive bacteria (86; 80–1112 mg/kg). Consistent with the MNM-culture results, the level of histamine was significantly higher in muscles of sardine than in muscles of mackerel.

The median (40 mg/kg) and IQR (49–106 mg/kg) levels found in mackerel were in agreement with the legal level of histamine (100–200 mg/kg) in histidine-rich fish placed in the markets during their shelf life (EFSA, 2011; FAO/WHO, 2012, 2018). In sardine, the median level of histamine (109 mg/kg) was also within the legal range, whereas the IQR (104–1094 mg/kg) warns for an elevated production of histamine in the muscles of sardine. The higher prevalence of HPB and levels of histamine among sardine as compared with mackerel agree with our observation that the sardine was caught locally in Egypt and sold unchilled as a sign of being fresh, whereas mackerel was imported frozen and sold chilled in the markets. In this aspect, it is claimed that the HPB is part of the fish gut and gill microbiome; once the fish dies, the HPB invade the muscles and start transformation of histidine to histamine (Kim *et al.*, 2003; Dewall *et al.*, 2006; Singh *et al.*, 2012). Exposure of the dead fish to a temperature >4°C for an extended period of time increases the activity of the HPB and fastens histamine accumulation, while keeping the fish chilled immediately after catching prevents this process (Ferrario, *et al.*, 2012; Wongsariya *et al.*, 2016). Another source of HPB to the fish could be contamination from water or postcatch due to improper hygienic measures. There is a mounting evidence that the human gut microbiota are histamine producing (Feng *et al.*, 2016; Pugin *et al.*, 2017). Therefore, we examined the presence of HPB in hand swabs from the fish vendors ($n = 20$); all the human samples were MNM-culture positive. Like in fish, *Enterobacteriaceae* predominate among the human isolates; the most common species found in the vendors and fish collected from the same markets were *C. freundii*, *E. cloacae*, *E. aerogenosa, K. pneumoniae*, and *A. baumannii*. In addition, the close relatedness between the *hdc* gene carried by fish and human strains isolated from the same market suggests a possible bidirectional transmission of the bacteria, which might occur during handling or butchering of the fish (Kim *et al.*, 2003; FAO/WHO, 2012, 2018). Furthermore, the high relatedness of the *hdc* gene among the same genus of *Enterobacteriaceae* (*R. planticola*) as well as different genus such as *Erwinia* spp. and *E. aerogenes*, is consistent with the suggestions that the *hdc* gene in the Gram-negative bacteria might originate from a common ancestor, which was exposed to some variations and deletions during the evolutionary divergence (Takahashi *et al.*, 2003; Hattouri and Seifert, 2017). Taken together, we showed the occurrence of HPB associated with histamine production in muscles of retail sardine and mackerel in Egypt. The sardine samples showed a relatively high level of histamine than the legal limit, possibly due to temperature abuse. Histamine was found in fish muscles that contain *hdc*-gene-negative bacteria, but in lower levels than in muscles with *hdc*-gene-positive bacteria. The close relatedness between the *hdc* gene extracted from the fish isolates and those from the human isolates warns for possible transmission of the HPB between the vendors and the fish. This recommends the necessity for regular inspection of the fish markets to ensure proper storage of retail fish and application of hygienic measures.

Disclosure Statement

The author(s) declare no competing interests.

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