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Microbial Quality of Raw Aquacultured Fish Fillets Procured from Internet and Local Retail Markets†

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ABSTRACT

The microbial quality of raw fillets of aquacultured catfish, salmon, tilapia, and trout was evaluated. A total of 272 fillets from nine local and nine Internet retail markets were tested. Mean values were 5.7 log CFU/g for total aerobic mesophiles, 6.3 log CFU/g for psychrotrophs, and 1.9 log most probable number (MPN) per gram for coliforms. Differences in these microbial levels between the two kinds of markets and among the four types of fish were not significant ($P > 0.05$), except that Internet trout fillets had about 0.8-log higher aerobic mesophiles than did trout fillets purchased locally. Although *Escherichia coli* was detected in 1.4, 1.5, and 5.9% of trout, salmon, and tilapia, respectively, no sample had ≥1.0 log MPN/g. However, *E. coli* was found in 13.2% of catfish, with an average of 1.7 log MPN/g. About 27% of all fillets had *Listeria* spp., and a positive correlation between the prevalence of *Listeria* spp. and *Listeria monocytogenes* was observed. Internet fillets had a higher prevalence of both *Listeria* spp. and *L. monocytogenes* than did those fillets purchased locally. *L. monocytogenes* was present in 23.5% of catfish but in only 5.7, 10.3, and 10.6% of trout, tilapia, and salmon, respectively. *Salmonella* and *E. coli* O157 were not found in any sample. A follow-up investigation using catfish operation as a model revealed that gut waste exposed during evisceration is a potential source of coliforms and *Listeria* spp.

Fresh fish fillets are highly perishable retail food items, and their bacteriological quality is a concern to the food industry and consumers *(8, 12).* Fresh fish and their production environment have been revealed as possible sources of foodborne pathogens, such as *Listeria monocytogenes, Salmonella enterica,* and *Escherichia coli* O157:H7 *(2, 13, 14, 23, 28, 34).* Common fish processing practices (e.g., cutting, eviscerating, and skinning) could result in contamination of fillets with undesirable microorganisms. The industry has adapted various food protection programs in addition to microbial testing to assure the freshness and safety of fish fillets. Products are considered unacceptable when excessive microbial counts or certain foodborne pathogens are found *(16, 32).*

In the United States, many food items (including raw fish) can be marketed directly to consumers by producers or distributors across the country through the Internet. This business approach satisfies consumers' desires to obtain perceived high-quality products directly from farms or production facilities by mail delivery *(29, 30, 33).* This alternative system of selling has expanded in recent years, but food safety information related to products in this emerging market is lacking. Other than a previous microbiological survey on sprouting seeds *(19),* we are unaware of any other reports of microbial data from food items sold via the Internet.

This study was designed to evaluate the bacteriological quality of four common types of raw fish fillets sold at local (central Virginia) and through Internet (U.S.) retail markets. A follow-up investigation on catfish processing also was conducted to help interpret results obtained from the commercial product evaluation.

MATERIALS AND METHODS

Commercial samples. Four types of raw (nonfrozen) fish fillets (catfish, salmon, tilapia, and trout) from nine local (central Virginia) and nine Internet (nationwide) retail venders were purchased in duplicate during fall 2006 and again in summer 2007 (Table 1). With a few original venders unavailable in 2007, a total of 272 (144 in 2006 and 128 in 2007) commercial fillets were obtained for this study. Internet samples were shipped by venders by commercial overnight or second-day delivery services, and local samples were transported in prechilled coolers by our laboratory staff. All samples were packaged in individual plastic bags, received at our laboratory in insulated containers packed with ice, and kept at 4°C for microbial testing within 24 h of arrival. To open sealed packages for sampling, one corner of each bag was spread with 70% ethanol and air dried before cutting with flame-sterilized scissors.

Sample analysis. To obtain sample portions for microbial testing, each fillet was cut into pieces (5 to 10 g per piece) with flame-sterilized scissors. For microbial enumeration, each sample portion (25 g, from multiple locations of a fillet) was homogenized with 225 ml of 0.1% peptone water in a laboratory blender (Masticator Silver, IUL Instruments, Barcelona, Spain) at high speed for 2 min. Appropriate dilutions of the samples were pour plated using standard method agar (unless otherwise noted, all media were purchased from Biotrace, Bothell, Wash.). Aerobic mesophile counts were determined after incubation at 35°C for 48 h, and psychrotroph plate counts were determined after incubation

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TABLE 1. *Market source and price of raw aquaculture fish fillets procured in 2006 and 2007*[a]

Vender	Catfish	Salmon	Tilapia	Trout
Local market (Virginia)				
1	√	√	√	√
2	√	√	√	√
3	√	√	√	
4	√	√	√	
5	√	√	√	
6	√		√	√
7		√	√	√
8	√	√		
9	√			√
10		√[b]	√	
11			√	√
12	√			
13		√[b]		
14				√
15				√
16				√
Price ($/kg)[c]	2.5 ± 0.2	3.9 ± 0.4	2.3 ± 0.2	2.0 ± 0.3
Internet market				
17 (Connecticut)		√	√	
18 (Louisiana)		√	√[b]	
19 (Ohio)	√[b]	√[b]	√[b]	√[b]
20 (Pennsylvania)	√	√	√	√
21 (North Carolina)	√	√	√	√
22 (North Carolina)				√
23 (New Jersey)	√	√	√	√
24 (New York)	√[b]	√	√	√
25 (Maryland)	√	√		√
26 (Maryland)	√			√
27 (Maryland)	√		√	
28 (South Carolina)	√	√	√	√
Price ($/kg)	5.1 ± 0.8	5.1 ± 0.4	4.2 ± 0.4	4.5 ± 0.3

[a] Samples were purchased from venders in fall 2006 and summer 2007. With duplicate samples obtained at each time of purchase, a total of 36, 32, 36, and 36 local samples and 32, 34, 32, and 34 Internet samples of catfish, salmon, tilapia, and trout fillets, receptively, were obtained for this study.

[b] Fish item available from the vender only in 2006.

[c] Mean ± SE of fish price (excluding tax or shipping and handling fee).

at 7°C for 1 week. Total coliform and *E. coli* counts were determined using the three-tube most-probable-number (MPN) method *(31).* After incubation for 24 to 48 h at 35°C, one loopful of culture from each lauryl sulfate tryptose broth culture tube that produced gas was transferred to brilliant green bile broth (BG) and *E. coli* broth (EC) containing 4-methylumbelliferyl-β-D-glucuronide (EC-mug). After incubation for 24 to 48 h, BG tubes with growth and gas production (at 35°C) confirmed the presence of coliforms, and EC-mug tubes with growth (at 45.5°C) and fluorescence under long-wave UV light (365 nm) indicated the presence of *E. coli (18).* Culture from all positive EC tubes were streaked to eosin–methylene blue agar, and purple colonies (with or without a green metallic sheen) were evaluated with API 20E test strips (bioMérieux, Hazelwood, Mo.) for *E. coli* confirmation.

Salmonella, E. coli O157, and *Listeria* detections were performed using AOAC-approved or performance-tested methods *(24–26).* For *Salmonella,* each sample (25 g) was macerated and preenriched in lactose broth (225 ml) at 36°C for 20 h, enriched in Rappaport-Vassiliadis broth at 42°C for 18 h, and postenriched in M broth at 36°C for 6 to 8 h. An enzyme-linked immunoassay was preformed with the *Salmonella* Visual Immunoassay test kit (Tecra, French Forest, Australia). For *E. coli* O157, each sample (25 g) was macerated and enriched in 225 ml of buffered tryptone soy broth with novobiocin and incubated at 42°C for 20 h before using the *E. coli* O157 Visual Immunoassay test kit (Tecra). For *Listeria* spp., each sample (25 g) was macerated in 225 ml of University of Vermont medium–modified *Listeria* enrichment broth. After incubation at 30°C for 24 h, 0.1 ml of the primary enrichment broth was transferred into 9.9 ml of Fraser broth for an additional enrichment at 30°C for 22 to 24 h before a *Listeria* Visual Immunoassay test kit (Tecra) was used. Fraser broth cultures with a positive response in the immunoassay were streaked onto Oxford *Listeria* agar for isolation. Up to five different colonies per isolation plate were identified to species using API *Listeria* kits (bioMérieux).

Follow-up processing evaluation. A follow-up investigation on catfish fillet production was conducted at the Randolph Farm (Virginia State University, Petersburg) to provide more information for interpretation of some results obtained from the commercial sample evaluation. Channel catfish (*Ictalurus punctatus*) were produced in aquaculture ponds following best management prac-

tices *(27).* Water for the ponds was pumped from the adjacent Appomattox River through screens before introduction into the ponds. Constant aeration maintained the dissolved oxygen above 3 ppm. A 32% crude protein floating catfish pellet was used (≤80 kg/ha/day) to feed the fish population (≤3,000 kg/ha). After an 18-month growth period, matured fish (~0.5 kg per head) were harvested and chilled on ice for immediate filleting on a thoroughly sanitized processing line composed of (i) a beheader (HV 25C V-Cut, Pisces Industries, Wells, Mich.), (ii) an eviscerator (DC 18C, Pisces Industries), (iii) a filletor (FS 200C, Pisces Industries), and (iv) a skinner (AFS 9800, Kemetec, Charlotte, N.C.). Individual samples were collected during processing, stored at 4°C in sterile polyethylene bags, and tested within 24 h following the protocols described for the commercial samples. Additional fillet samples were tested after 7, 14, and 21 days of storage at 4°C.

Statistical analysis. Log-transformed microbial (aerobic mesophile, psychrotroph, coliform, and *E. coli*) populations were analyzed with an analysis of variance, and prevalence of *E. coli* and *Listeria* were analyzed with the Spearman rank correlation coefficient using SigmaStat (version 3.0, SPSS Inc., Chicago, Ill.) software. Differences were considered significant at $P \leq 0.05$. For the evaluation of commercial samples, log-transformed values obtained from duplicate samples of each purchase were averaged before the analysis. For the processing evaluation, data are means and standard errors (SE) of three replications.

RESULTS AND DISCUSSION

Retail fillet evaluation. Locally purchased catfish, salmon, tilapia, and trout fillets had total aerobic mesophiles of 6.0 ± 0.2, 5.5 ± 0.3, 5.4 ± 0.4, and 5.6 ± 0.2 log CFU/g, respectively, psychrotrophs of 6.8 ± 0.3, 6.4 ± 0.3, 6.0 ± 0.4, and 6.3 ± 0.3 log CFU/g, respectively, and coliforms of 2.5 ± 0.2, 1.9 ± 0.3, 1.7 ± 0.3, and 1.8 ± 0.3 log MPN/g, respectively (Fig. 1). Internet-ordered catfish, salmon, tilapia, and trout had total aerobic mesophiles of 6.0 ± 0.3, 5.4 ± 0.2, 5.6 ± 0.3, and 6.3 ± 0.2 log CFU/g, respectively, psychrotrophs of 6.3 ± 0.3, 6.1 ± 0.2, 5.9 ± 0.5, and 6.9 ± 0.3 log CFU/g, respectively, and coliforms of 2.1 ± 0.3, 1.4 ± 0.2, 2.0 ± 0.3, and 2.0 ± 0.3 log MPN/g, respectively. Thus, aerobic mesophile, psychrotroph, and coliform levels were similar between local and Internet fillets except that trout from Internet venders had slightly higher aerobic mesophile counts (0.8 log CFU/g) than did those purchased locally ($P = 0.04$). The differences in the aerobic mesophile, psychrotroph, and coliform levels among the four types of fish were not significant ($P > 0.05$).

For raw fish fillets, aerobic mesophile counts often are used to indicate quality. Both the mean aerobic mesophile and coliform levels found in this study were within the broad ranges (6.9×10^3 to 1.9×10^8 CFU/g for aerobic mesophiles and <3 to 9.3×10^3 CFU/g for coliforms) reported previously *(3, 20).* Based on the limits established by the International Commission on Microbiological Specifications for Foods (ICMSF) *(16),* about 57.1% of local and 48.5% of Internet retail fillets tested in the current study had aerobic mesophile counts within the recommended range for good quality fresh fish (total counts ≤ 5×10^5 CFU/g), and 29.3 and 37.9% of local and Internet samples, respectively, were marginally acceptable (total counts of 5×10^5 to ≤10^7 CFU/g).

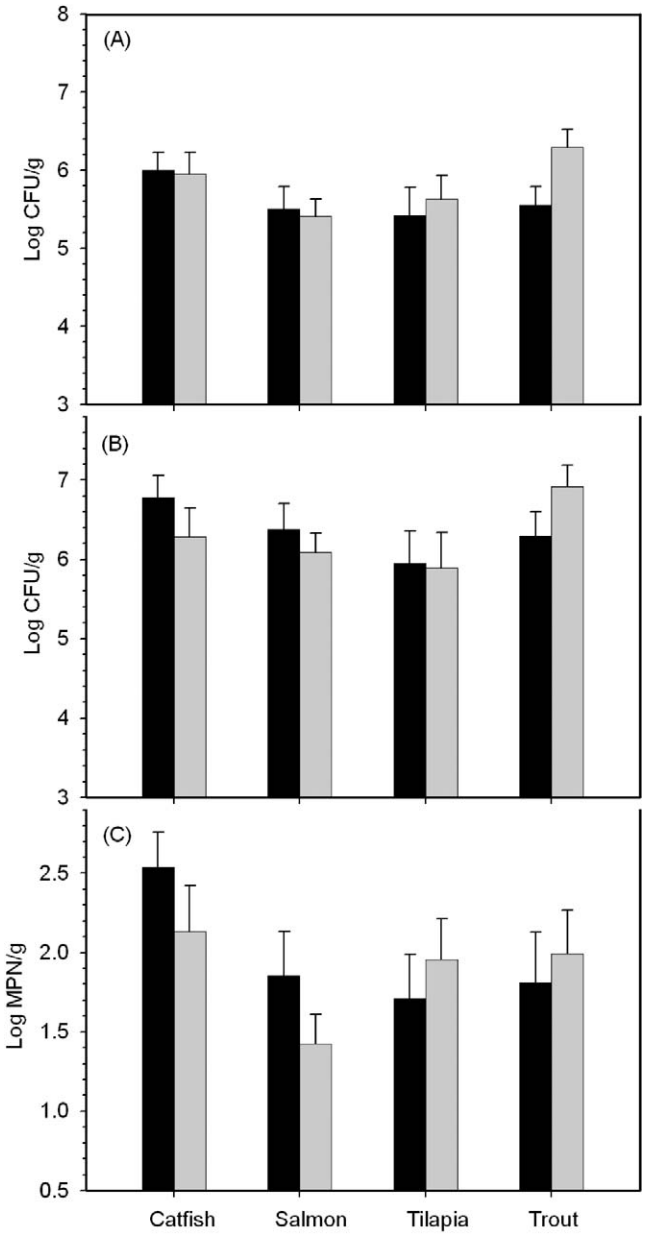

FIGURE 1. *Microbial levels in raw fish fillets procured from local (■) and Internet (□) retail markets. Data are the means ± standard errors* (n ≥ *16) of (A) aerobic mesophile, (B) psychrotroph, and (C) coliform populations.*

Further analysis revealed that fish in general purchased during summer 2007 had an average total coliform level of 2.2 log MPN/g, which is about 0.5 log higher ($P = 0.01$) than that of fish purchased in fall 2006 (data not shown). This difference was mainly due to local market fish; local trout and tilapia had 1.2 and 1.9 log MPN/g higher coliform levels, respectively, in summer 2007. One obvious contributing factor associated with the observed higher counts in summer is the warmer weather, which promotes bacterial growth. Previous studies on aquacultured fish also revealed higher levels of coliforms in fish and their production environment during warmer weather *(1, 9).*

E. coli ranging from 3 to 240 MPN/g was found in 15

TABLE 2. *Prevalence of bacterial contamination in raw fish fillets from retail markets*

Bacteria	Prevalence (%)[a]									
	Overall		Catfish		Salmon		Tilapia		Trout	
	Local	Internet	Local	Internet	Local	Internet	Local	Internet	Local	Internet
Coliforms	83.6	86.4	97.2	93.8	93.8	76.5	75.0	87.5	69.4	88.2
Escherichia coli	6.4	4.6	16.7	9.4	0.0	2.9	8.3	3.1	0.0	2.9
Listeria spp.	20.0	35.6	58.3	75.0	9.4	23.5	5.6	31.3	5.6	14.7
L. monocytogenes	9.3	15.9	22.2	25.0	9.4	11.8	2.8	18.8	2.8	8.8

[a] Prevalence was determined for samples obtained from local (central Virginia) or Internet (U.S.) markets in 2006 and 2007 (overall local and Internet sample numbers were 140 and 132, respectively; individual local or Internet fish sample numbers were ≥32). All fillets were negative for *Salmonella* and *E. coli* O157.

of the 272 tested retail fillets (9 local fish and 6 Internet fish) during the two sampling periods (Table 2). However, none of the fillets had an *E. coli* level exceeding the limit recommended by the ICMSF *(16)* for marginally acceptable quality (11 to ≤500 cells per g) and 97.8% of them met the committee's criteria for good quality fresh fish (≤11 cells per g). *E. coli* was detected in 1.4, 1.5, and 5.9% of trout, salmon, and tilapia fillets, respectively. The levels of *E. coli* in these positive samples were all <1.0 log MPN/g, with a combined (trout, salmon, and tilapia) level of 0.7 ± 0.1 log MPN/g. At no time did duplicate samples from any purchase test positive. For catfish samples, however, 9 (13.2%) of the 68 fillets had *E. coli* levels of 1.7 ± 0.3 log MPN/g. Both the prevalence and mean *E. coli* population in the positive catfish samples were higher than those observed for other types of fish. Six of the nine positive samples were from three cases in which duplicate samples both tested positive. These results indicate that fecal contamination is a more serious problem for catfish than for salmon, tilapia, or trout at retail markets. However, the levels of *E. coli* found in the positive local and Internet samples were not significantly different ($P > 0.05$). No significant correlation was found ($P > 0.05$) between the prevalence of coliforms and the prevalence of *E. coli* (Table 2). No *E. coli* O157 or *Salmonella* was detected in any sample. Similar recent studies have revealed *E. coli* but not *Salmonella* in aquacultured fish from conventional retail markets *(12, 17).* These results are an improvement in comparison to the data from earlier years, when the incidence of *Salmonella* in raw fish was significant *(14, 36).* Nonetheless, living fish muscle is not a natural habitat for *E. coli (4)*; thus, the sporadic presence of *E. coli* observed in this study is a clear indication that fecal contamination still occurs in modern fish processing and/or handling operations in the United States.

About 27% of the 272 fish fillets tested positive for *Listeria,* and *L. innocua, L. monocytogenes, L. welshimeri, L seeligeri,* and *L. ivanovii* were isolated from 41, 34, 22, 6, and 3 samples, respectively. In a recent study, Chou et al. *(6)* found similar isolates of *Listeria* on raw catfish fillets collected from processing plants. Chou et al. also found higher prevalences of *L. monocytogenes* (37.4%), *L. innocua* (21.4%), and *L. welshimeri* (20.6%) than other *Listeria* species. Instead of *L. monocytogenes* being the most prevalent species, in our study *L. innocua* was the most prevalent species in both catfish and aquacultured fish fillets at retail markets. In a market survey in northern Greece, fishery products also more frequently contained *L. innocua* than *L. monocytogenes (22).* The data shown in Table 2 indicate a positive correlation ($r = 0.95$, $P = 0.00$) between the prevalence of *Listeria* in general and the prevalence of *L. monocytogenes.* The highest prevalence of *Listeria* contamination was associated with retail catfish. *L. monocytogenes* was present in 23.5% of catfish compared with 10.6, 10.3, and 5.7% of salmon, tilapia, and trout. Previous reports also indicated a wide range of *L. monocytogenes* prevalence (0 to 50%) in fish samples with a relatively low occurrence in raw salmon when compared with catfish *(6, 13, 34, 35, 37).*

Fish fillets ordered from the Internet had higher prevalence of both *Listeria* spp. and *L. monocytogenes* contamination when compared with fish purchased from local stores (Table 2). Only 2.8% of the tilapia from local stores was contaminated with *L. monocytogenes* in comparison to 18.8% of the tilapia from Internet venders. When results obtained in autumn 2006 or summer 2007 were analyzed independently (data not shown), the overall fish fillets ordered from the Internet still had higher prevalences of *Listeria* spp. and *L. monocytogenes* contamination than did products from local stores. Although the Internet market offers great convenience and many choices of raw fish products, the results of this study suggest that there was a greater chance of getting *L. monocytogenes*–contaminated products through this ordering route.

Although *E. coli* and *L. monocytogenes* were found in 15 and 34 of the 272 fillet samples, respectively, only 6 samples tested positive for both *E. coli* and *L. monocytogenes.* Thus, *E. coli* was not a reliable indicator organism for *L. monocytogenes* contamination in raw fish fillets.

Processing evaluation. Catfish fillets had a higher prevalence of bacterial contamination than did any other fish tested during the retail evaluation. Thus, an experimental catfish operation with steps typical for all types of fish filleting was used as a model to determine potential sources of processing contamination.

Water in contact with catfish in our aquaculture pond and processing plant had no detectable coliforms or *E. coli* (<3 MPN/g) and was free of *Listeria* (<1 cell per 25 g). Coliforms and *Listeria* were found in all three gut waste

samples but not in skin waste during processing. Samples of fillets were contaminated with total aerobic mesophiles at 2.5 ± 0.3, 6.6 ± 0.5, 9.0 ± 0.3, and 9.1 ± 0.1 log CFU/g, psychrotrophs at 2.3 ± 0.2, 7.3 ± 0.3, 9.2 ± 0.2, and 9.1 ± 0.2 log CFU/g, and coliforms at 0.5 ± 0.0, 0.5 ± 0.0, 0.7 ± 0.1, and 4.6 ± 0.3 log MPN/g when stored at 4°C for 1, 7, 14, and 21 days, respectively. The results show that raw fillet products, even produced and kept under ideal experimental conditions, can quickly develop microbial populations. Previous studies of aquacultured catfish, cod, and trout also indicate that aerobic mesophile and psychrotroph levels in fillets maximize at around 9.0 log CFU/g after storage at 4°C for about 2 weeks *(10, 15).* The results of the current study indicate that some coliforms slowly adapted to refrigeration temperature; however, their growth was insignificant before the fillets spoiled from overgrowth of aerobic mesophiles or psychrotrophs.

Catfish fillets generally have a shelf life of 5 to 8 days during refrigerated storage *(8).* The results of our study corroborate this estimation. Refrigerated catfish fillets (4°C) at day 7 had an average aerobic mesophile count of 6.6 log CFU/g, which is near the acceptable limit (≤7.0 log CFU/g) recommended by the ICMSF *(16).* These results and the information obtained from the above retail survey emphasize the importance of prompt delivery and consumption of both Internet and locally purchased fish fillets.

All fillet samples tested negative for *Listeria* spp. on days 1 and 7; however, one of three samples tested positive on both days 14 and 21. *Listeria* isolates from all positive samples were exclusively identified as *L. innocua.* Finding *L. innocua* during the later weeks of the refrigerated storage study is not surprising because psychrotrophic bacteria (including *Listeria* spp.) are capable of survival and proliferation in refrigerated fish muscle *(10, 21).* In a processing plant contaminated with *L. monocytogenes,* Autio et al. *(5)* did not find *L. monocytogenes* in trout fish skins. Several other researchers have identified antimicrobial properties in fish skin components *(7, 11).* The influence of these antimicrobials on the survival and effective detection of *Listeria* spp. and other microbial contaminants associated with fish skin deserves further investigation. No *E. coli* or *Salmonella* was detected in any sample from our processing evaluation. Unlike *Listeria* organisms, typically *E. coli* O157 and *Salmonella* are linked to fecal contamination from warm-blooded animals and thus can be minimized through good aquaculture and manufacturing practices.

Internet fish fillets in general are marketed at higher prices than those sold locally even before the addition of shipping and handling fees. In our study, the average Internet fish price was about 1.8 times higher than that offered by local retailers (Table 1). By paying higher prices, some consumers may believe that they are getting products of superior quality. However, there is no scientific evidence showing that Internet fish are of better microbial quality than locally purchased products. The results of our study suggest that Internet fish products are equally as likely or more likely to be excessively contaminated with bacteria, including *L. monocytogenes,* than are locally purchased fillets. Thus, careful handling and cooking of raw fillets by consumers, regardless of the market source, is required to prevent foodborne illness. Continued research and extension efforts are needed to support the healthy development of this emerging market.

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