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Isolation of *Salmonella*, *Yersinia*, and *Vibrio* Bacteria from some Seafood Sold in Retail Stores in the Libyan Cities of Sousse and Hamama

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ABSTRACT

Seafood is an important source of essential nutrients, including protein, omega-3 fatty acids, phosphorus, and zinc, contributing to public health. However, it can also harbor human pathogens that cause various diseases. Libya, with its extensive Mediterranean coastline, possesses a rich marine resource, making the monitoring of pathogenic contamination in seafood crucial. In this study, 120 samples of oysters, sepia (*Sepia officinalis*), crabs, and shrimp were collected from retail outlets in Sousse and Hamama in 2023 and transported to the Veterinary Teaching Hospital at Omar Al-Mukhtar University for microbiological analysis. Standard laboratory procedures were used to detect *Salmonella*, *Yersinia*, and *Vibrio* species, and all work was conducted following ethical guidelines. The occurrence of bacterial isolates varied among seafood types, with *Salmonella* detected in 20% of oysters, 16% of crabs, 8% of sepia, and 12% of shrimp. *Yersinia* isolates were found in 32% of oysters, 32% of crabs, 28% of sepia, and 36% of shrimp, while *Vibrio* species were detected in 24% of oysters, 8% of crabs, 20% of sepia, and 20% of shrimp. These findings highlight the notable prevalence of pathogenic bacteria in seafood sold at retail outlets in Eastern Libya and emphasize the need for effective food safety measures to minimize contamination and protect public health.

1. INTRODUCTION

Seafood products are affected by factors such as the location of fishing, the surrounding environment, microbial exposure to the product, and practices that affect the product in terms of processing, handling, and preparing the product for cooking before consumption. All these aspects must be taken into account, to make this seafood fit for consumption and a product free of intestinal pathogens. In most cases, edible marine organisms are caught from the sea and processed directly without the addition of any chemicals or food additives. All fish are considered to be of high nutritional value to humans, as they contain protein, phosphorus, zinc, omega-3 and unsaturated fatty acids, with a small amount of fats that are harmful to human health. (Mahmoud, Saleh, & Alsadi, 2020).

40% of developing countries derive 35% of their protein annually from seafood and fish species that generally live off their coasts, particularly crustaceans and mollusks such as shrimp, lobster, and clam (Abbass et al, 2025). Marine organisms are also a source of protein, omega-3, and harmless fats, which are important in many medical conditions, especially anemia and goiter, as they provide about 18% of protein from animal sources, especially sheep and goats, which are consumed by the majority of the world's population (Bufarwa et al., 2025). Seafood or products are more susceptible to bacteria, which are the cause of many intestinal diseases and gastrointestinal infections (Mahmoud, Saleh, & Gaballah, 2024). The possibility of contamination occurs because the fishing areas are a polluted area where there is a large percentage of disease-causing bacteria, and all of this depends on the microbial diversity, environmental factors, and fishing areas (Zafir, Almardi, Alorfi, Saleh, & Hamad, 2023). Salmonella is the main and dangerous cause of many food poisonings. However, it is difficult to determine whether pollution of marine organisms has occurred in their aquatic environment due to contamination of the fishing area with wastewater or during their handling, storage and marketing (Hamad & Saleh, 2019). *Yersinia bacteria* are microbes that cause contamination of marine organisms and many fish, including molluscs and crustaceans, and thus cause humans to suffer from many diseases and intestinal symptoms such as colic and diarrhea when eating these seafood and fish, knowing that signs of spoilage are not clear on these foods. (Carson & Wilson, 2009). It is known that the bacterium *Vibrio cholerae* is widely spread in salty sea water, especially on the beaches of the Mediterranean, and it is the main cause of intestinal inflammation associated with eating fish or seafood (Soutiyah et al., 2023). This study aims to detect contamination of certain types of seafood with *Salmonella*, *Yersinia*, and *Vibrio* bacteria, and to implement strict preventive measures and health requirements to avoid contamination of seafood with pathogens detected during storage, transportation, and preparation for cooking.

2. METHOD

Experiment design:

This study was conducted in 2024 AD, and included collecting a sample of 120 seafood samples. 30 samples collected for each type from retail stores in coastal areas such as Sousse and El Hamama these stores were chosen by simple random sample in the city of Al-Bayda Libya. Ethical approval was obtained from the central laboratory at the Veterinary Teaching Hospital, and all procedures followed ethical guidelines and regulations.

Sample collection

A sample of total of 120 seafood specimens, including oysters, crabs, *sepia officinalis*, and shrimp, with 30 specimens each, were collected from retail stores in coastal areas such as Sousse and El Hamama in the city of Al-Bayda Libya. Samples were collected under refrigerated conditions, placed in a separate, numbered, sterile plastic bag, and transported in a dedicated icebox to the central laboratory at Omar Al-Mukhtar University in Al-Bayda, Libya. These samples were transported in accordance with health requirements and sample transport recommendations to ensure sterile conditions. Upon arrival, all samples were examined bacteriologically to isolate and identify *Salmonella*, *Yersinia*, and *Vibrio*, the study subjects. After examination, the samples were transferred to the central laboratory at Omar Al-Mukhtar University in Al-Bayda, Libya, which is one of the specialized laboratories, where they were processed within 15 minutes of purchase.

Preparing fish samples for microbiological examination:

The outer surface of the targeted specimens for study was sterilized with sterile alcohol and set on fire, and 30 g of them were taken into a well-sterilized mixing device containing 461 ml of sterile peptone water [0.2%]. All contents were homogenized rapidly at 15,000 rpm for 2 full minutes. The mixture was left for 20 minutes at room temperature in a sterile atmosphere.

Microbiological examination of samples:

Isolation of *Salmonella bacteria*:

The World Health Organization recommends a specific method for isolating *Salmonella* bacteria from food (Abdalnaser et al., 2025). This method involves using a sterile swab to inoculate MacConkey agar and *Salmonella-Shigella* agar plates, as well as a 10 ml selenite broth tube. The plates and tubes are then incubated at 37.0°C for 24 hours. Once incubated, the plates are checked for the growth of suspected *Salmonella* colonies.

The relevant selenite broth tube is then used to inoculate new MacConkey agar and *Salmonella-Shigella* agar plates, which are also incubated for 24 hours at 37°C. Any suspicious colonies are further tested by plating on Trypticase Soy Agar plates and incubated at 37°C for 24 hours. Finally, isolated organisms were identified using regular biochemical tests, and their final identity is confirmed using BioMérieux strips (Abduljalil et al., 2024; Biasizzo et al, 2005).

Isolation of *Yersinia* spp.:

Yersinia species were identified and isolated using the following procedure: first, the supernatant was mixed with Trypticase Soya Broth (TSB) and left to incubate for 24 hours. Next, an enrichment was streaked onto MacConkey's plates and incubated for 1-2 days. Lactose-negative colonies were then streaked onto CIN Agar plates and incubated for another 1-2 days. Afterward, susceptible colonies that showed a "bull's eye" appearance were isolated and sub cultured onto nutrient agar. Finally, colonies were identified using basic chemical methods, and samples were then cultured on nutrient agar. (Adebayo et al., 2012; Alawy et al, 2015).

Isolation *Vibrio* spp.

To isolate *Vibrio* spp. from fish, first enrich the sample in Alkaline Peptone Water (APW) at 37°C for 24 hours. Then, streak two loopfuls of the enrichment culture onto Thiosulfate citrate bile salts sucrose (TCBS) agar plates and incubate at 37°C for 24 hours. After that, perform a series of characterization tests including Gram staining, motility test, catalase test, cytochrome oxidase activity test, Triple sugar iron test, ornithine, arginine, lysine, valine, leucine dehydrolase test, nitrate reduction test, gelatin hydrolysis test, starch hydrolysis test, and fermentation tests for glucose, lactose, mannitol, maltose, and sucrose. These tests will help identify the specific type of *Vibrio* bacteria present in the sample.

Data Analysis:

Descriptive statistics were used to present the results in the form of counts and percentage, using Microsoft Excel data sheath. SPSS 23.0 software was used. Univariate analysis was done in the form of frequency tables, bivariate analysis (cross tables). Chi-square was used for bivariate analysis. Probability level (P value) < 0.05 considered significant.

3. ETHIC APPROVAL

This study did not involve any human participants or live animals. The research was limited to microbiological examination of seafood samples (oysters, sepia, crabs, and shrimp) collected from retail markets. Ethical approval was obtained from the Central Laboratory at the Veterinary Teaching Hospital, Omar Al-Mukhtar University, Libya. All laboratory procedures were conducted in accordance with institutional ethical guidelines and biosafety regulations.

4. RESULT

Table 1 the Significance of the Relationship in the Model

Seafood type		Bacterial isolate		
		Salmonella spp. Positive	Yersinia spp. Positive	Vibrio spp. Positive
Oyster	N/30	5	8	6
	%	16.7%	26.7%	20.0%
	P	0.335	1.000*	0.385
Crab	N/30	4	8	2
	%	13.3%	26.7%	6.7%
	P	0.748	1.000*	0.236
Sepia	N/30	2	7	5
	%	6.7%	23.3%	16.7%
	P	0.513	0.634*	0.772
Shrimp	N/30	3	9	5
	%	10.0%	30.0%	16.7%
	P	1.000	0.634*	0.772

N Number of positive isolates among the specimens of type of seafood % Percentage of positive isolate among the type of seafood P value for difference of the bacterial positive isolate distribution for the seafood type in comparison to all other types together.

* Pearson Chi square. † Fisher exact test. Note: all results were statistically insignificant at 95% level of significance.

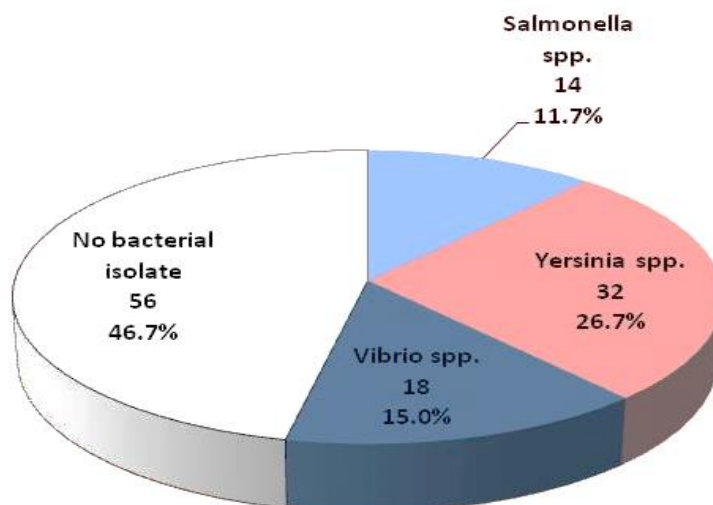


Fig. 1 Distribution of the isolates of investigated bacterial pathogens among the total study sample of the seafood.

Table 2 Distribution of the isolates of *Salmonella* spp. (serotypes) among different types of the seafood.

Seafood type		Salmonella spp. Isolate		
		S. typhimurium	S. enteritidis	S. Haifa
Oysters	N/30	2	1	2
	%	6.7%	3.3%	6.7%
Crabs	N/30	1	1	2
	%	3.3%	3.3%	6.7%
Sepia	N/30	0	0	2
	%	0.0%	0.0%	6.7%
Shrimp	N/30	1	1	1
	%	3.3%	3.3%	3.3%

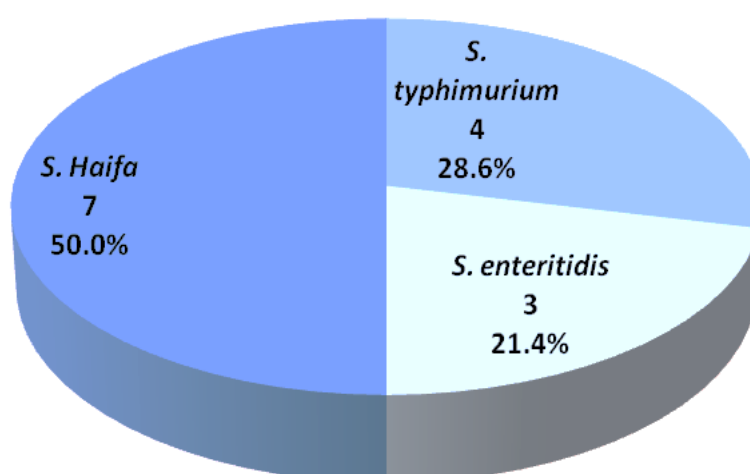


Fig. 2 Distribution of the isolates of *Salmonella* spp. serotypes among the total study sample of the seafood.

Table 3 Distribution of the isolates of *Yersinia spp.* (serotypes) among different types of the seafood.

Seafood type		Yersinia spp. Isolate			
		Y . enterocolitica	Y. frederiksenii	Y. ruckeri	Y. intermedia
Oysters	N/30	2	4	1	1
	%	6.7%	13.3%	3.3%	3.3%
Crabs	N/30	3	1	3	1
	%	10.0%	3.3%	10.0%	3.3%
Sepia	N/30	2	1	2	2
	%	6.7%	3.3%	6.7%	6.7%
Shrimp	N/30	2	3	2	2
	%	6.7%	10.0%	6.7%	6.7%

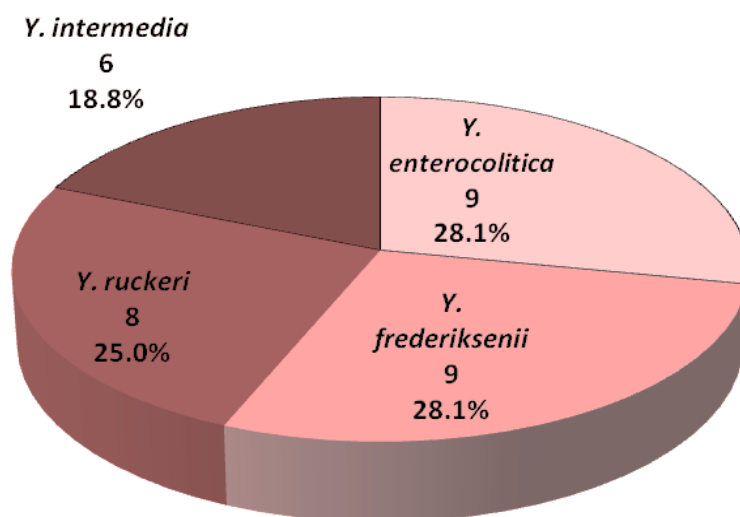


Fig. 3 Distribution of the isolates of *Yersinia spp.* serotypes among the total study sample of the seafood.

Table 4 Distribution of the isolates of *Vibrio spp.* (serotypes) among different types of the seafood.

Seafood type		Vibrio spp. Isolate	
		V. cholera	V. parahaemolyticus
Oysters	N/30	3	3
	%	10.0%	10.0%
Crabs	N/30	1	1
	%	3.3%	3.3%
Sepia	N/30	4	1
	%	13.3%	3.3%
Shrimp	N/30	3	2
	%	10.0%	6.7%

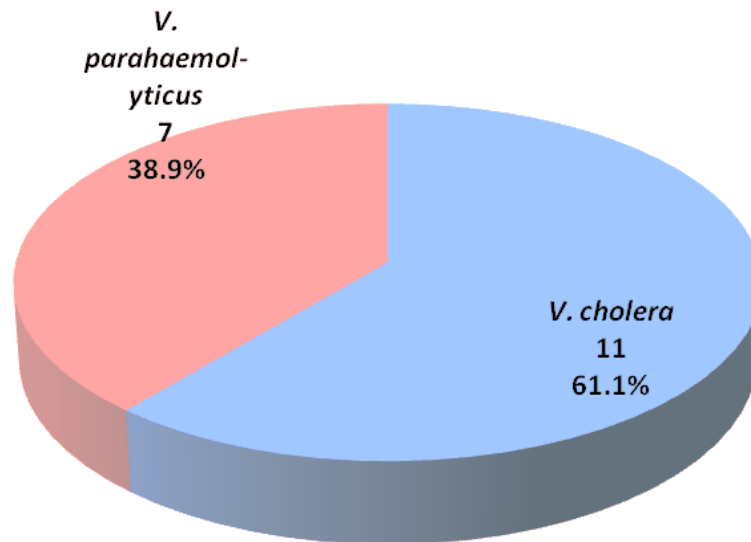


Fig. 4 Distribution of the isolates of vibrio app. serotypes among the total study sample of the seafood.

5. DISCUSSION

Fresh seafood is an important source of nutrients for humans, as it contains protein, zinc, phosphorus, omega-3, and unsaturated fatty acids that are important for human health. In their environments, seafood is exposed to a number of disease-causing microorganisms, some of which affect the consumer. Most seafood contamination occurs in the environment where it is caught, or during food preparation and handling. Cross-contamination can occur between food handling and preparation, with most of the pathogenic bacteria targeted by this study being transferred from infected raw seafood and/or contaminated surfaces used in preparation, and/or from utensils used to contaminate healthy seafood and fish. (Aidara et al., 2018; Ali et al, 2004). All data in Table 1 indicate that the percentage of pathogenic bacteria isolated from the tested samples of the targeted seafood species was 16.7% *Salmonella*, 30% of *Yersinia*, and 20% of *Vibrio* in oysters, shrimp and oysters respectively. Thus, all the results obtained did not agree with Libyan and international standards. All data included in the study (Table 2) indicate that the incidence of *Salmonella* isolated from all examined samples of the targeted seafood species in the study was 16.7%, 13.3%, 6.7%, and 9.9% in oysters, crabs, squid, and shrimp. respectively. This result did not agree with the international standard specifications for seafood, which stipulate that the marine organisms eaten must be completely free of the salmonella microbe in 30 grams (Adebayo et al., 2012; Alawy et al, 2015).

Almost similar results were obtained from another study conducted in 2024. The results revealed that the incidence of *Salmonella* isolated from the examined samples of seafood species was 16, 8, 4, and 8% in *oysters*, crabs, sepia, and shrimp, respectively. Serotyping of *Salmonella* isolated from the examined seafood samples revealed the detection of *S. Enteritidis* and *S. Typhimurium* and *S. Haifa* (Zafir et al., 2024; Soutiyah et al, 2023). Serological diagnosis of *Salmonella* isolates showed that *S. Enteritidis* was isolated from oysters, crabs, squid, and shrimp, respectively, with an infection rate of 9.9% each. *S. Typhimurium* was also isolated from oysters, crabs, squid, and shrimp, respectively, with an infection rate of 13.3% each. *Salmonella* haefe was also isolated from oysters, crabs, and brown shrimp, respectively, with an incidence rate of 23.4% for each. All of these results are consistent with the researchers' report that *Salmonella* haefe is the most frequently isolated *Salmonella* serotype, which also indicates that *Salmonella* haefe is the most prevalent serotype in seafood and fish. (Carson et al., 2009; DePaola et al 2003; Feldhusen et al, 2000). Similar lower results were obtained in Slovenia where *oyster* samples were contaminated with one (0, 5%) strain of *Salmonella* (Carson et al., 2009; Feldhusen et al, 2000). In Nigeria, *Salmonella* was isolated from two seafood samples (10%) (Saeed et al., 2025; Hussein n et al, 2022). In Egypt, 4 samples (10%) of oysters were found (Carson et al., 2009; Feldhusen et al, 2000), and in Tunisia 4 samples (25%) were contaminated with *Salmonella*, and lower results were found in seafood products (0% *Salmonella*) in Poland (18). Much higher results were obtained: in 100 samples of fish and shellfish from markets and from fish landing areas in France, *Salmonella* was detected in 70% of the fish, 59% of the shrimp, and 30% of the shellfish samples (Carson et al., 2009; Feldhusen et al, 2000).

Salmonella contamination on seafood farms may arise from poor water quality, farm runoff, and fecal contamination from wild animals or livestock. *Salmonella* contamination of shrimp may also be caused by high stocking densities, high water temperatures, poor retail distribution, marketing, handling, and preparation practices. These factors should be carefully monitored and addressed to reduce the risk of *Salmonella* contamination in seafood farms (Carson et al., 2009; Fernández et al, 2007).

Table No. (3) displays data on the percentage of infections with *Yersinia* species in 30 samples for shrimp, sepia, crab, and oysters. The study found that different proportions of *Yersinia* species were present in these samples. *Yersinia enterocolitica* was found to be the most common, being found in 6.7% of shrimp, 6.7% of sepia, 10.0% of crabs, and 6.7% of oysters. *Yersinia fredericksonii* was found in 3.3% of crabs, *Yersinia rockeri* in 3.3% of oysters, and *Yersinia intermedia* in 3.3% of crabs. Similar outbreaks have been reported previously; In Italy, *Y. enterocolitica* has been isolated from 1% of frozen crustacean or mollusc samples (Carson et al., 2009; Feldhusen et al, 2000). *Y. enterocolitica* has been identified in fish and shellfish in both terrestrial and aquaculture environments (Bufarwa et al., 2022; Mahmoud et al, 2024). A study was conducted to analyze the presence of *Yersinia enterocolitica* in 288 samples of raw seafood, including shrimp, mussels and cephalopods, obtained from different retail markets in Algeria. The results showed that the prevalence of *Y. enterocolitica* was 2.9% in the samples, although the prevalence varied among different types of seafood (Mustapha et al., 2025; Saeed et al, 2025). According to a study conducted in 2022, the incidence of *Yersinia* infection found in samples of seafood species was relatively high. Oysters, crabs, sepia, and shrimp showed the presence of *Y. enterocolitica*, *Y. fredericksonii*, *Y. ruckeri*, and *Y. intermedia* by 44, 36, 24, and 28%, respectively. Identification of these bacteria was confirmed by biochemical tests (Heinitz et al., 2003; Ripabelli et al, 2004). *Yersinia enterocolitica* is a pathogen that can cause diarrhea, even in developed countries. The fact that they are becoming more common globally is concerning from a public health point of view, because it indicates a higher risk of food-borne contamination. This is a problem that must be addressed to protect public health. *Vibrio parahaemolyticus*

It is a type of bacteria responsible for causing gastroenteritis in humans, especially when they consume undercooked seafood and do not adhere to health requirements in trading. *Oysters* have been found to contain *Vibrio spp.* In concentrations up to 100 times higher than those found in surrounding water, due to its ability to filter and concentrate these bacteria (Carson et al., 2009; Saleh et al, 2023; Li et al, 2018).

Table 4 shows that the percentage of *Vibrio* bacteria (which includes *V. parahaemolyticus* and *V. cholera*) is 10.0% in oysters, 3.3% in crabs, 13.3% in sepia and 10.0% in shrimp. Another almost identical study of seafood species found that the incidence of *Vibrio* isolates from the samples examined was 12%, 9%, 10%, and 4% in oysters, crabs, sepia, and shrimp, respectively. Isolates obtained from *Vibrio* were identified by biochemical tests that confirmed the presence of *V. parahaemolyticus* and *V. cholera* (Bufarwa et al., 2024; Fernández et al, 2000; Hamad et al, 2019).

Vibrio parahaemolyticus It is a type of bacteria known to cause infections in people who consume seafood. These bacteria are straight or curved, Gram-negative, motile, oxidase-positive, facultative anaerobes that occur naturally in marine waters. It is commonly found in shrimp, shellfish and fish and can cause serious illness. The three main diseases caused by *Vibrio parahaemolyticus* are *gastroenteritis*, wound infections, and septicemia (Bufarwa et al., 2025; Deepanjali et al, 2005). Its percentage reached 10% in oysters, 3.3% in crabs, 3.3% in sepia, and 6.7% in shrimp. Based on these results, it can be concluded that *Vibrio* bacteria are present in various marine species, and appropriate measures must be taken to ensure their safety for consumption. These results were expected due to handling contamination during processing or during storage and retail in *supermarkets*, as well as via rodent droppings such as rat and mouse droppings containing *Salmonella*, *Yersinia* and *Vibrio* species that contaminate foods directly or indirectly and cause cross-contamination

6. CONCLUSION AND RECOMMENDATION

Through the results presented in this study on seafood products that are contaminated with enteric bacteria and coliforms and contain serotypes of salmonella, vibrio, and pathogenic yersinia, which makes these marine organisms unfit for consumption and harmful to the health of consumers. accordingly, the following recommendations should be taken into account: these creatures must be caught from areas free of any human or animal waste. the utensils and equipment for handling must also be clean and made of materials that can be disinfected and cleaned. this seafood must be kept at lower temperatures than when they were caught and before marketing. they must be placed in sterile boxes because they are a food item that spoils quickly. it contains crushed ice. all persons working in restaurants or handlers must undergo periodic medical examinations. the competent health authority must participate in setting laws, regulations and health requirements for dealing with, marketing and storing fish and marine organisms.

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