

Food-borne Pathogens in Seafood

Maliha Afreen¹, Ilknur Ucak^{1*}

¹Nigde Omer Halisdemir University, Faculty of Agricultural Sciences and Technologies, Nigde, Turkey

*Corresponding author: ilknurucak@ohu.edu.tr

ORCID: 0000-0002-9701-0824

Abstract

Nowadays food poisoning is a major social and scientific issue. Food poisoning is caused by those foods which looks normal when we see it, smells normal and even tastes normal when we eat it. Only special scientists can analyze food quality by checking its all-sensory parameters. Food poisoning caused by foodborne pathogens. These pathogens can be bacteria, viruses, fungi and algae. Pathogens mostly attack on those foods which stored in humid environment, high temperature and which have more water content. According to these conditions' seafood is more susceptible food for contamination. Seafood can be contaminated by pathogens at any stage from harvesting to fork. Sometime fish can become poisonous even in water by some bacteria's or by some algal toxins. Most common pathogens which involve in seafood poisoning are *Vibrio*, *Salmonella*, *Listeria*, *Shigella*, *Staphylococcus*, *Clostridium* and *Escherichia coli*. Poisoning diseases which can occur are scombroid poisoning, amnestic shellfish poisoning and diarrheic poisoning, etc. Some viruses also involved in seafood born infections. These infections can be avoided by using proper cleanliness and care in handling fish. In this review the most common food-borne pathogens in fish and fish products are discussed.

Keywords: Seafood poisoning, food-borne pathogens, contamination, infection, bacteria, virus

Review article

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INTRODUCTION

What is Pathogen?

Biological agents (viruses, bacteria, parasites) that can cause foodborne infections are called foodborne pathogens. When two or more than two cases of same disease occur due to eating of same food is called foodborne disease epidemic (CDC, 2012). Pathogens were divided into groups on the basis of illness brutality in 1986. In 2000 International Commission on Microbiological Specification for Foods revised this division of pathogens, which is given in below table. Some indicator organisms can be used to reveal the occurrence of those foodborne pathogens which are difficult to detect. 'Indicator organisms' are linked with organisms of intestinal origin.

Another term used for the detection of environmentally similar foodborne pathogens is ‘index organism’ that was proposed by Ingram in 1977 as a marker. Microbial indicators are mostly used for checking the food quality and safety. Ideal food safety indicator should: a) be easily and quickly detectable, b) be easily differentiated from other members of the food flora, c) whose numbers perfectly relate with concerned pathogen, d) keeping growth rate and requirements similar to those of the pathogen. Common indicator organisms are coliforms *E. coli*, Enterobacteriaceae and Fecal *Streptococci*.

Table 1. Some pathogens and effects of hazards

Effects of hazards	Pathogens
Moderate, direct, limited spread, death rarely occurs Moderate, direct, potentially extensive spread, death or serious sequelae can occur. Considered severe, direct Categorization of common foodborne pathogens (ICMSF, 1986)	<i>B. cereus</i> , <i>C. jejuni</i> , <i>Cl. perfringens</i> , <i>S. aureus</i> , <i>Y. enterocolitica</i> , <i>Tuenia saginata</i> , <i>Toxoplasma gondii</i> , Pathogenic <i>E. coli</i> , <i>S. enteritidis</i> and other <i>Salmonellae</i> other than <i>S. typhi</i> and <i>S. paratyphi</i> , <i>Shigellae</i> other than <i>Sh. dysenteriae</i> , <i>L. monocytogenes</i> , <i>Cl. botulinum</i> types A, B, E and F, hepatitis A virus, <i>Sh. dysenteriae</i> , <i>S. typhi</i> and <i>S. paratyphi</i> A, B, and C, 7: spiralis
Proposed updated categorization (ICMSF, 2000) Food poisoning organisms causing moderate, not life threatening, no sequelae, normally short duration, self-limiting Serious hazard, incapacitating but not life-threatening, sequelae rare, moderate duration Severe hazard for general population, life-threatening, chronic sequelae, long duration Severe hazard for restricted populations, life-threatening, chronic sequelae, long duration	<i>B. cereus</i> (including emetic toxin), <i>Cl. perfringens</i> type A, Norwak-like viruses, <i>E. coli</i> (EPEC, ETEC), <i>S. aureus</i> , <i>V. cholerae</i> non-01 and non-0139, <i>V. parahaemolyticus</i> <i>C. jejuni</i> , <i>C. coli</i> , <i>S. enteritidis</i> , <i>S. typhimurium</i> , <i>shigellae</i> , hepatitis A, <i>L. monocytogenes</i> , <i>Cryptosporidium parvum</i> , pathogenic <i>Y. enterocolitica</i> , <i>Cyclospora cayatanensis</i> Brucellosis, botulism, <i>EHEC (HUS)</i> , <i>S. typhi</i> , <i>S. paratyphi</i> , tuberculosis, <i>Sh. dysenteriae</i> , aflatoxins, <i>V. cholerae</i> O1 and O139 <i>C. jejuni</i> O: 19 (GBS), <i>C. Perfringens</i> type C, hepatitis A, <i>Cryptosporidium parvum</i> , <i>V. vulnificus</i> , <i>L. monocytogenes</i> , EPEC (infant mortality), infant botulism, <i>Ent. sakazakii</i>

Food poisoning is caused by those foods which looks normal, gives normal smell and even normal in taste. In this condition consumer could not estimate that this food has pathogens and when they ingest it to exceeding limit of infectious dose, they become ill. As a result, it is difficult to know which food was the exact reason of food poisoning. Many organisms can cause food poisoning, their incubation and disease time periods significantly change from one organism to another organism.

Table 2. Microorganisms and incubation periods

Microorganism	Incubation period	Duration of illness
<i>Aeromonas</i> species	Unknown	1- 7 days
<i>C. jejuni</i>	3-5 days	2- 10 days
<i>Escherichia coli</i> types		
ETEC	16-72 hours	3-5 days
EPEC	16-48 hours	5- 15 days
EIEC	16-48 hours	2-7 days
EHEC	72-120 hours	2- 12 days
Hepatitis A	30-60 days	2-4 weeks
<i>Listeria monocytogenes</i>	3-70 days	Variable
Norwalk-like virus	24-48 hours	1-2 days
Rotavirus	24-72 hours	4-6 days
<i>Salmonellae</i>	16-72 hours	2-7 days
<i>Shigellae</i>	16-72 hours	2-7 days
<i>Yersinia enterocolitica</i>	3-7 days	1-3 weeks

Food poisoning is divided into two types on the basis of their causing factors and their method of causing disease. Infections contain those microorganisms which growing in the human intestinal tract, while Intoxications contain those microorganisms which can produce toxins in the food or also when passing through the intestinal tract. These groups are very useful to identify the paths of food poisoning. Aquatic environment naturally contains pathogens or these can produce due to polluted water. This water pollution produced by excreted fertilizers, human sewage, and due to animal farms. Aquaculture products become contaminated from bacteria, viruses, parasites and biotoxins (Huss et al., 2003). Most common aquatic pathogens are *Escherichia coli*, *Salmonella*, *Shigella*, *Yersinia*, *Brucella* and *Edwardsville*.

Why Fish is Important in Poisoning?

Seafood is a best nutritional food containing proteins, unsaturated fatty acids, vitamin D, iodine and selenium. Ingestion of fish is very important for health, during pregnancy and early growth stages (Emmett et al., 2013) and it is also useful for cardiac patients (Zarrazquin et al., 2014). Usage of Seafood has increased in U.S regularly from 1980 to 2006. Seafood is very health but it is also a main carrier for spreading of many bacterial diseases. Food poisoning diseases in humans caused by different pathogenic bacteria and these are normally transferred from fin fish, shell fish and other sea food products (Okuda et al., 1997).

Pathogens which are main causing agents of infection in fish are *Vibrio cholerae*, *Vibrio parahaemolyticus*, *Salmonella* sp., *Staphylococcus aureus*, *Listeria monocytogenes*, *Yersinia enterocolitica*, *Campylobacter jejuni* and *Escherichia coli* (Venugopal et al., 1999). Seafood which is getting from coastal areas is the main source of pathogenic microorganisms due to joined population (Kumar et al., 2005).

Antimicrobials which are officially used for seafood protection in aquaculture farming are therapeutics and prophylactics. As usage of antimicrobials increasing, their extra residues accumulated in water and become dangerous for aquatic species (Marshall and Levy, 2011). When unapproved antimicrobials such as chloramphenicol, nitrofurantoin, etc. are used it becomes dangerous for human health. Extra use of antibiotics for elimination of bacteria can improve antibiotic resistance in aquatic pathogens (Cabello, 2006). Antimicrobials such as β -lactams, streptomycin's, and aminoglycosides can add in aquaculture through water solution or through feed and these can be a major cause of contamination in aquaculture.

Scientists reported that genes of antimicrobial resistance can transfer horizontally from microorganisms to human through food chain (Heuer et al., 2009). This transfer can be of two types: one is direct transmission and other is indirect transmission. If this transfer done by ingestion of zoonotic bacteria which itself carries antimicrobial gene then it is called direct transmission (Heinitz et al., 2000). If aquatic pathogens transfer antimicrobial resistance gene to human pathogen then it is transferred to humans is called indirect transmission (Heuer et al., 2009). Zoonotic infections are of two types: a) when it caused by direct contact with aquatic animals is called topically acquired infection; b) when it caused by eating of undercooked food.

BACTERIAL INFECTIONS

When humans come in direct contact with bacterias like *V. vulnificus*, *M. marinum*, *S. iniae*, *E. tarda*, *Aeromonas hydrophila* (Lehane & Rawlin, 2000) and *Erysipelothrix rhusiopathiae* (Brooke and Riley, 1999; Harada et al., 2011) from fish, shellfish or crustaceans, through damage of vertebera (Janda & Abbott, 1993). These infections are mostly occurred in immunodeficient patients. Those persons who have routinely skin contact with fish, live in humid climate and at temperature which is favorable for bacterial growth, are also more susceptible for infection. Principal species of *Vibrio* (*Vibrio vulnificus*, *V. parahaemolyticus*, *V. damsela* and *V. cholerae*) are incriminate in human infections. (Dryden et al., 1989). This infection can be lethal when it is caused by *V. vulnificus* (Austin, 2010).

Edwardsiella tarda

A systemic disease 'fish gangrene' caused by *Edwardsiella tarda*, catfish disease known as 'emphysematous putrefactive' and 'red disease of eels' caused by *Edwardsiella septicaemia*.

Streptococcus iniae

A zoonotic pathogen *Streptococcus iniae* in fresh & marine water produce infection in aquatic species mostly in the tilapia and hybrid striped bass (Agnew and Barnes, 2007). It is a Gram-positive bacterium, which produced severe infection in humans.

Mycobacterium marinum

It is an acid-fast rod-shaped bacterium that produces lethal and severe infection in many fish species in fresh and marine waters all over the world (Evans et al., 2009; Nichols et al., 2004).

Mycobacterium marinum usually causes loss of scales, loss of appetite, discoloration, apathy, exophthalmus, slimeless skin, ulcers and produces tumors in many parts of body, even leads to death (Gauthier & Rhodes, 2009). *M. marinum* has ideal growth temperature of 30°C. *M. marinum* causes granulomatous swelling of the skin, swelling of dermal tissues, tendon sheaths of fingers and hands, Offensive septic arthritis and osteomyelitis in less immune hosts (Jernigan and Farr, 2000; Lahey, 2003).

Grouping on the Basis of Etiologic Agents

Pathogenic microorganisms are the main factors for water contamination. This contaminated water will produce infectious food. These pathogenic infections have different causes and prevalence's. On the basis of their prevalence, etiologic agents categorized pathogens into two groups of major and minor concern. Microbial agents of major concern in seafood include: norovirus, *Vibrio*, and *Salmonella*. Minor agents of concern include hepatitis A virus (HAV), *Shigella*, *Listeria monocytogenes*, *Clostridium botulinum*, *Staphylococcus aureus* and *E. coli* (Hadler, 1991).

SIGNIFICANT GROUP of PATHOGENS

Norovirus

It is known as “RNA calicivirus” highly transmissible infection causing in humans. Norovirus has two Geno groups GI and GII, which can cause infection in human (Woods et al., 2016; Bazzardi et al., 2014). This infection known as the principal reason of digestive problems without bacteria due to eating of undercooked shellfish and oysters (Woods et al., 2016). The main sources of norovirus were Mussels and clams (Bazzardi et al., 2014; Terio et al., 2010) prawns, crabs, and finfish (Anbazhagi and Kamatchiammal, 2010) spider, gooseneck and barnacles (Sala et al., 2008). Symptoms of this infection are vomiting, nausea, abdominal cramps, headache and fever (Iwamoto et al., 2010).

Vibrio

Vibrio is the main cause of human food poisoning. It spreads in the main portion of fresh and marine waters so it is mostly secluded from seafood specifically from shellfish. *Vibrio* species are rod-shaped, Gram-negative bacterium. It is facultative anaerobic, halophilic, curved and non-spore forming. It is motile with the help of flagella. Their habitat is in seashore areas, water deposits, and nearly all animals and plants of seaside containing this pathogen (Schaferer et al., 2011; Thompson et al., 2005).

Vibrio has 65 species, from which only 3 species *V. parahaemolyticus*, *V. cholera* and *V. vulnificus* are mostly responsible for food borne infections (Sudha et al., 2012; Gopal et al., 2005). Other *Vibrio* spp. which can cause disease in humans infrequently are *V. mimicus*, *V. fluvialis*, *V. damsella*, *V. hollisae*, *V. alginolyticus*, *V. furnissi*, *V. metschnikovii* and *V. cincinnatiensis*.

Vibrio vulnificus

Vibrio vulnificus is seafood borne pathogen. *V. vulnificus* has been divided into three subtypes on the basis of their biochemical properties are following:

- Biotype 1 in crustaceans and humans (Tison et al., 1982)
- Biotype 2 in eels (Tsai et al., 1990)
- Biotype 3 in tilapia (Bisharat et al., 1999). It causes wound infection in humans during dealing with fish, also causes liver illnesses and hemochromatosis which leads to death (Rajapandian et al., 2009).

Vibrio vulnificus is a zoonotic offensive pathogen (Austin, 2010). When an injured person comes into contact with contaminated seawater, fish or shellfish caused primary wound infections even in low number of pathogens (Dalsgaard et al., 1996; Oliver, 2005) followed by fasciitis necroticans (Dijkstra et al., 2009; Kuo et al., 2007) and even leads to death (Ralph & Currie, 2007).

Vibrio parahaemolyticus

In many Asian countries *V. parahaemolyticus* usually known as cause of seafood-borne infection (Deepanjali et al., 2005). It is a halophilic gram negative, motile, oxidase positive, straight or curved rod- shaped, facultative anaerobic bacteria that occur naturally in marine water. These are native microflora of aqueous environment and mostly cause severe illnesses in penaeid shrimp, shellfish and fish (Nelapati et al., 2012). Gastroenteritis, wound infections, and septicaemia are three major diseases caused by *V. parahaemolyticus* (Nair et al., 2007).

Vibrio cholera

V. cholerae and its relative *V. mimicus*, both bacteria's can occur in both marine and pond water. They can grow in laboratory without using sodium chloride so these are known as unusual *Vibrio* species (Nishibuchi and DePaola, 2005; Parveen and Tamplin, 2013). *V. cholerae* produce Cholera, a severe chronic diarrhea. Centre for Disease Control and Prevention has classified Cholera as category B bioterrorism (WHO, 2008).

Salmonella

Seafood is a main source of *Salmonella* contamination (D'Aoust et al., 2001). This pathogen excretes in fresh water reservoirs and in seashore reservoirs through fecal contamination. Salmonellae are facultative anaerobic and Gram-negative bacteria. This bacterium is small, rod-shaped, catalase positive, oxidase negative, and can move with flagella. It causes diarrhea and inflammatory reaction. This infection starts symptoms after 12 to 72 h of ingestion of contaminated food. *Salmonella* infections are mostly caused by eating poorly cooked seafood like finfish, shrimps and crustaceans (NACMCF, 2008).

MINOR GROUP of PATHOGENS

Hepatitis A Virus

It is non-enveloped virus belongs to the Hepatovirus genus and the Picornaviridae family. Natural habitat of Hepatitis A virus are humans and other Vertebrates (ICTV, 2012). Symptoms of this disease can appear in 2 to 3 weeks (WHO, 2016) and it can also reach 45 days (Richards, 2013). Children have no symptoms; most patients have mild symptoms but these can be severe in old or less immune persons. Symptoms of this disease are loss of appetite, fever, abdominal pain, jaundice and diarrhea (Ghasemian et al., 2016). HAV is a critical disease with low death rate. Main reasons of this disease are usage of fecal polluted water, ingestion of contaminated seafood, and improper cleanliness (Richards, 2013).

Listeria Monocytogenes

It is a non-spore forming, Gram-positive bacteria with rod shaped. It is facultative food borne pathogen microorganism of humans and animals (Dhama et al., 2013). It can survive at refrigerated temperature, at low pH and in high salty environment (Gandhi and Chikindas, 2007). It is a lactic acid producing bacteria. *Listeria monocytogenes* are universal bacteria of all soil, water, animal and plant environments because it can adjust in broad range of temperature and PH (Jay et al., 2005). A foodborne disease also involving seafood caused by *Listeria* spp. is called Listeriosis. This disease can be transmitted to humans by eating packed seafood products (Miya et al., 2010). Symptoms of this disease are chilling, nausea, fever, gastroenteritis etc. followed by septicemia, meningitis, encephalitis, abortion and even death (Barbuddhe et al., 2008). Pregnant women, immunodeficient and elderly people have more chances of listeriosis (Parihar et al., 2008; Das et al., 2013).

Shigella Spp.

It is rod-shaped, Gram-negative, immobile, oxidase-negative and non-lactose fermenting bacteria. *Shigella* species are categorized into four types due to its serology antigen. Group. A involve *Sh. dysenteriae*, Group. B involve *Sh. flexneri*, Group. C involve *Sh. boydii*, and Group. D involve *Sh. sonnei* (O'Connell et al., 1995; Lampel, 2005). Symptoms of shigellosis are fever, abdominal pain, tenesmus, and bloody diarrhea. This bacterium attack on intestinal cells, can exist in stomach acid, due to its multi-virulence aspects (Wang et al., 2011).

Clostridium botulinum

It is spore forming, anaerobic, Gram-positive bacillus bacteria. *C. botulinum* produces neurotoxin which can contaminate food. Human botulism has four transmission types involving fish, wound, child, adult and immunodeficient persons (CDC, 2014b). Symptoms of this disease are dizziness, fatigue, double vision, difficulty in swallowing and speaking, and weak muscles (Aberoumand, 2010). This infection can cause by eating canned products of seafood. Spores and toxins of *Clostridium botulinum* are mostly occurred in flounder, cod, white fish, rock fish and in smoked fish. (Iwamoto et al., 2010).

Staphylococcus aureus

It is catalase positive, Gram +Ve and chemotrophic bacterium. *S. aureus* enterotoxins are causing foodborne infection but it is not done by raw seafood (NACMCF, 2008). Worldwide most prevalent causes of gastroenteritis caused by eating staphylococcal contaminated food (Jablonski and Bohach, 2001).

Escherichia coli

All over the world *Escherichia coli* serogroup O157 is one of the most important evolving foodborne pathogens (WHO, 1997). *E. coli* can produce many enterotoxins so it can mostly become the reason of food infections (Sharma et al., 2005). *E. coli* O157:H7 infection can cause severe bloody diarrhea (Haemorrhagic Colitis, HC), chronic complications such as Haemolytic Uraemic Syndrome (Kumar et al., 2001).

SEAFOOD POISONING ASSOCIATED WITH BIOTOXINS

Seafood considered as 'healthy food, but it can also cause food poisoning produced by toxins. These toxins are originating from toxic algae and also from bacteria, for example: Puffer fish poisoning and scombroid poisoning.

Scombroid Poisoning

This poisoning caused by ingestion of contaminated seafood, especially of the Scombridae species. Scientists thought that histamine is main causing factor of this poisoning (Ucak and Gokoglu, 2020; Ucak et al., 2019; Ucak et al., 2018; Yerlikaya et al., 2014). This histamine produced from histidine which is naturally present in fish. Bacterial enzymes histidine decarboxylase converted histidine to histamine biogenic amine. Bacteria's which are commonly involved in this process are *Proteus*, *Enterobacter*, *Serratia*, *Citrobacter*, *Clostridium*, *Vibrio*, *Acinetobacter*, *Pseudomonas*, and *Photo bacterium* (Lopez-Sabater et al., 1996; Tsai et al., 2004).

Fish species which can mostly cause scombroid poisoning are Tuna, mackerel, bonito, sardines, anchovies, herring and pilchards (Scoging, 1998; Bartholomew et al., 1987). Symptoms of this disease are rashes on neck, face and upper chest, diarrhea, sweating, nausea, headache, abdominal pain, dizziness, metallic taste and burning in mouth (Scoging, 1998; Attaran and Probst, 2002).

Toxic Diseases Caused by Marine Algae

40 species of marine algae, out of more than 5000 species produce effective toxins. These toxins are produced in the form of cysts that can rest inactive for years. As algae found proper growing conditions, algae cell will multiply rapidly (Daranas et al., 2001). This toxin may then enter in the aquatic organisms which can be eaten by fish or sometime directly enter in fish. In this way Shellfish can become enough poisonous to cause disease in person within 1 day.

Amnesic shellfish (ASP) Poisoning

ASP produces neurotoxins that are water-soluble. Major toxic compound is domoic acid which gradually stored in gastrointestinal mucosa (Hess et al., 2001; Daranas et al., 2001). Toxin is produced by dinoflagellates and by diatoms an effective promoter for increasing calcium permeability and glutamate receptors, which eventually ends with cell death. Domoic acid found in many types of fish, like mackerel, jack smelt, albacore, anchovies, sardines, sanddabs and in krill (Lefebvre et al., 2002). This disease can cause neurological problems (confusion, memory loss, disorientation, seizure, and coma) and gastrointestinal diseases (vomiting, diarrhea, abdominal pain) (Todd, 1993). Neurological symptoms occur within 2 days, while gastroenteritis symptoms occur within 1 day.

Azaspiracid Poisoning

This poisoning is caused by *Protoperidinium*. This genus is universal hence azaspiracids can exist in shellfish of all over northern Europe. This poisoning was confirmed first in Netherlands in 1995. Symptoms of this disease are nausea, vomiting, severe diarrhea and stomach cramps, with disease interval up to 5 days (James et al., 2002).

Diarrheic shellfish (DSP) Poisoning

DSP toxins are of four types.

- Okadaic acid
- Dinophysis toxins
- Pectenotoxins
- Yessotoxins

Two types of toxins like okadaic and sometime dinophysis toxins are strong protein phosphatase inhibitors, hence they promote the accumulation of phosphate in many intracellular proteins and promote tumor formation. Dinoflagellates produce pectenotoxins and yessotoxin (Burgess and Shaw, 2001). Symptoms of this disease are gastrointestinal disorders, including nausea, diarrhea, and abdominal cramps with chills, headache and fever. This disease can occur within half hour to a few hours after eating contaminated seafood (Daranas et al., 2001).

Paralytic shellfish Poisoning

Paralytic shellfish poisoning (PSP) is lethal. Incubation period of disease differs from 15 min to few hours less than 10 hours. Symptoms include tingling or numbness around the lips, which spreads to the face and neck, a prickling sensation in the fingertips, headache, fever, nausea, vomiting and diarrhea. In the worst cases Death can occur due to breath obstruction (Anonymous, 2002). Toxins of this disease occur in mussels, clams, oysters, scallops, abalone, gastropods, crustacea, crabs and lobster (Oikawa et al., 2002). Toxins of this disease secreted from dinoflagellates.

Ciguatera Poisoning

Ciguatoxins are produced by algae found on tropical coral reefs (Yasumoto, 2001) and are mostly occur in toxic coral reef fish, including moray eels, groupers, snappers, barracuda, parrot fish and mullet. In this disease patients have sensory problems, that touching cold water gives pain as an electric shock (Ting and Brown, 2001). Symptoms of this poisoning are diarrhea, vomiting, muscle pain and itching, Prickling of the lips, tongue and throat, Headache Severe pain in arms, legs, impaired vision. Neurological disorders can last for many years but this disease is not lethal (Yasumoto, 2001).

CONCLUSION

Seafood is a main carrier of many bacterial disease's transmission in humans. This contamination can occur by bacteria, viruses and many other algal toxins. Seafood pathogens naturally live in both marine and fresh water environments. These pathogens can contaminate seafood at any stage of harvesting and processing. Seafood demand increasing worldwide but due to contamination of fish and seafood products, fish exporters facing loss. To overcome this problem proper handling and care should apply during fish processing from harvesting to table.

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