


A review on microbial contamination cases in Tunisian coastal marine areas

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ABSTRACT

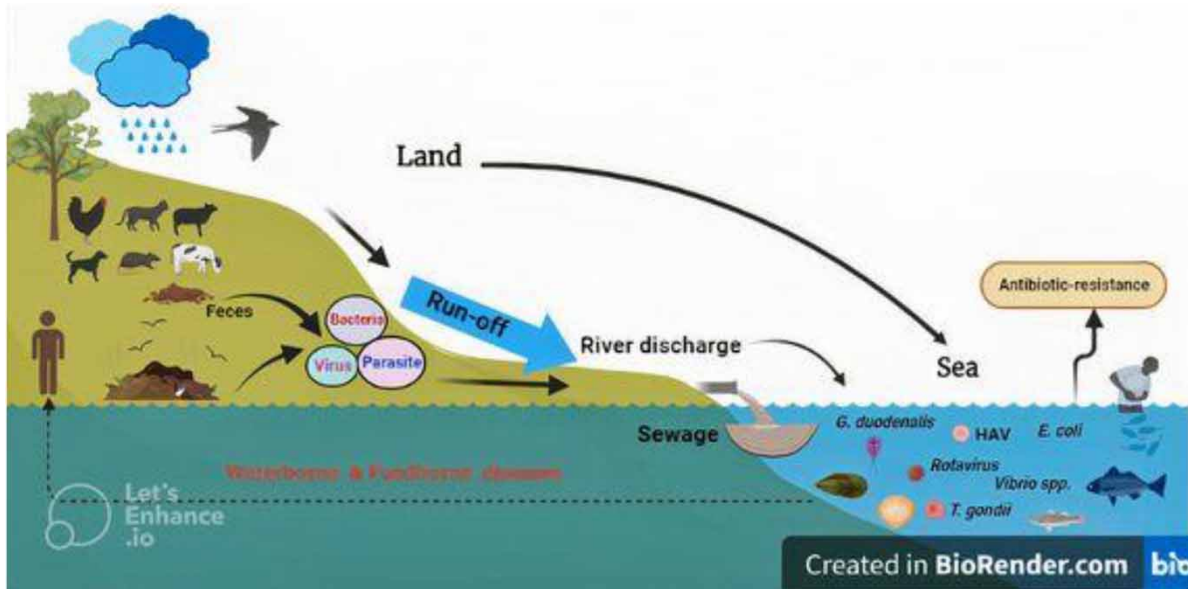
Microbial pollution in marine environments is one of the critical issues with regard to the sanitary status of recreational activities and seafood harvesting due to a potential contamination by pathogenic microorganisms. This review's objectives were to identify instances of bacterial, viral and protozoan parasite pollution in the Tunisian coastal region and to make recommendations for further research. Fecal indicators such as *Escherichia coli* and *Salmonella* spp. were detected in samples of clams and mussels. Vibrionaceae species were also recorded in seawater, sediment, fish and clams in different sites from north to south with the dominance of *Vibrio alginolyticus*. Bivalve mollusks collected from the Tunisian coast have been revealed to harbor viruses as well as protozoan parasites. Furthermore, the isolation of multi-drug-resistant bacterial strains from Tunisian coastlines proves the significant spread and circulation of antibiotic resistance caused by the massive use of antibiotics. In conclusion, we suggest intensive monitoring and cutting-edge wastewater treatment technologies to enhance seawater quality and preserve the biodiversity of aquatic life. Rapid detection techniques for the most important pathogenic microorganisms in seafood and seawater must be also developed to reduce human health risk.

Key words: bacteria, contamination, parasite, Tunisian coast, virus

HIGHLIGHTS

- Monitoring the microbial quality of the Tunisian coast environment is crucial.
- Wastewater discharge was the main cause of microbial pollution and the spread of multi-resistant strains.
- Improvement of wastewater treatment procedure is highly recommended to reduce the pollutant loads to the sea.
- More investigations should be conducted especially for viral and parasitic contamination.

GRAPHICAL ABSTRACT



1. INTRODUCTION

Contamination of coastal areas by pathogens transmitted from land to sea poses a real hazard to human health and aquatic organisms since these areas are frequently used for recreational and aquaculture activities. Microbial contamination in the marine environment can occur in all marine biota anytime sewage from human or animal origin is discharged into coastal waters (Ospar Commission 2009). Pathogens including bacteria, parasites and viruses can concentrate in coastal, estuaries and beach sands, infecting and causing illness and/or death in both humans and animals. Moreover, these pathogens can cause ecological alterations to coastal habitats by harming marine coastal communities wildlife (Shapiro *et al.* 2018). These pathogenic microorganisms mainly derived from human or animal feces can be transported from upstream sources to estuarine and coastal waters, especially during heavy rain or flood events (Malham *et al.* 2014). Bacteria, viruses and parasites from humans and animals, transmitted to the coastal environment mainly attached to fine particulate matter, can affect bathing water quality and can accumulate in filter-feeding shellfish. A wide range of pathogens has been responsible for waterborne and shellfish-borne illnesses (Gonçalves *et al.* 2018).

Special consideration should be given to the presence of fecal indicator bacteria for their possible direct impact on human health particularly in areas used for shellfish production or recreation. In general, bacterial level contamination is highly affected by wastewater discharges to the seacoast. The impacts of microbiological pollution depend on weather (rainfall and light), turbidity and hydrodynamics (Ospar Commission 2009). Therefore, a growing number of human bacterial infections have been associated with the recreational and commercial uses of marine resources. According to the World Health Organization, waterborne diseases such as cholera, typhoid and hepatitis cause approximately two million deaths per year in some rural areas of third-world countries, most of which are in children under five (WHO 2000).

More interest towards the assessment of the microbiological quality of coastal marine environments has been attributed since the outbreak of gastro-enteric diseases and skin dermatitis episodes in bathers (Zappalà *et al.* 2012). The assessment of seawater microbiological quality is conventionally performed through the search of indicator microorganisms such as coliform bacteria, *Escherichia coli* and intestinal *enterococci*. The presence of pathogenic bacteria like *Salmonella* spp. and *Escherichia coli* were also evaluated in shellfish and fish (Kim *et al.* 2017; Atwill & Jearnsripong 2021). Moreover, the genus *Vibrio* is endemic in marine and estuarine ecosystems and some species might cause gastrointestinal diseases or, in some cases, septicemia (Oliver & Kaper 1997).

In the last 20 years, medical communities have been faced with the apparition of multidrug-resistant species due to the massive use of antibiotics (Sundaramanickam *et al.* 2015). Besides, antibiotics usage in aquaculture is well known and this practice can cause the spread of antibiotic residues in the marine environment (Okeke *et al.* 2022). The use of the marine

and coastal environment for fishing, recreational, commercial and other activities has increased several fold. Since 2006, some studies reported the occurrences of antibiotic-resistant bacteria in coastal waters and marine sediments (Ben Kahla-Nakbi *et al.* 2006, 2009; Harakeh *et al.* 2006; Akinbowale *et al.* 2007). Ben Kahla-Nakbi *et al.* (2006) showed that strains of *Vibrio alginolyticus* isolated from the internal organs of diseased gilthead sea bream (*Sparus aurata*) and sea bass (*Dicentrarchus labrax*) cultured in two fish farms on the eastern Tunisian coast were resistant to at least two antimicrobial agents. All the tested strains were resistant to ampicillin. However, 91.17% were resistant to nitrofurantoin and 35.29% to tetracycline.

In addition, enteric viruses are very common in seawater where they are rejected in large amounts, and shed by infected people. They are transmitted into the environment through the discharge of treated and untreated wastes. They represent a risk to human health with a low infectious dose generally 1–10 viral units. They are responsible for many cases of nonbacterial gastroenteritis, respiratory infection, conjunctivitis and hepatitis, causing high morbidity and mortality in immunocompromised and in immunocompetent individuals worldwide. Among the enteric viruses, hepatitis A virus (HAV) is considered one of the most serious food-borne viruses that can be transmitted through raw and or undercooked shellfish (Fusco *et al.* 2017, 2019). Other enteric viruses, such as norovirus (NoV), aichivirus (AiV), astrovirus (AsV), adenovirus (AdV) and rotavirus (RoV) can also potentially cause human illnesses (Cioffi *et al.* 2020).

Furthermore, protozoan parasites are discharged, dumped or carried in the runoff, from the massive amounts of humans and their pet's feces to estuaries and coastal waters. These pathogens have the potential to cause diseases in humans and animals, posing public health risks. Zoonotic protozoa such as *Giardia*, *Cryptosporidium* and *Toxoplasma* are important parasites that can be transmitted to the marine environments and accumulated by shellfish consumed by humans and marine mammals and infect a wide range of marine animal hosts. Along the Mediterranean seacoasts, several edible and inedible shellfish have been found contaminated by such parasites (Giangaspero *et al.* 2005, 2014; Gómez-Couso *et al.* 2005, 2006; Melo *et al.* 2006; Putignani *et al.* 2011; Aksoy *et al.* 2014).

Several studies in Tunisia have been performed to assess bacterial and viral contamination in the marine environment, but protozoan parasites have received less attention. According to the original research by Ghozzi *et al.* (2017), shellfish from the Tunisian shore may contain zoonotic protozoa like *Giardia*, *Toxoplasma* and *Cyclospora*.

Monitoring microbial contamination levels is therefore critical, not only from an ecological but also from a public health standpoint, since marine ecosystems provide a significant food supply for the human population (Belton *et al.* 2014).

This review aims to summarize the incidence of bacteria, viruses and parasites found in marine coast areas throughout the previous decades in order to evaluate the state of microbial contamination of Tunisian coasts. It is intended to be a reference synthesis and support for further investigations.

2. BACTERIAL CONTAMINATION

Fecal contamination of coastal regions poses a serious risk to both aquatic life and human health. The presence of pathogenic bacteria in bathing area has a significant impact on human health and marine-borne infections. Direct discharges of treated and untreated wastewater, farming drainage, livestock and domestic animals are important sources of pathogens to coastal marine ecosystems. Microbial quality of water has typically been evaluated by monitoring fecal indicator bacteria, such as *Escherichia coli*, intestinal enterococci and fecal coliform bacteria.

Investigations of bacterial contamination in Tunisian shoreline water and sediment have received little attention in the literature. The findings are summarized in Table 1.

The results of recent research by Zaafrane *et al.* (2022) in Monastir Bay demonstrated the absence of fecal coliform bacteria in water masses throughout all sampling periods, confirming the absence of any impact from human waste pollution, especially in the north part of the Bay. Not far from Monastir Bay, a similar study carried out in the Rejiche coast (Mahdia) by Alibi *et al.* (2021) to examine the microbial properties of seawater and sediment revealed that most probable number (MPN) of *Salmonella*, *Vibrio* and *total coliform* were below the Tunisian standard limits in all sites. Nevertheless, *Enterococcus faecalis* was detected in both seawater and sediment and exceeded the accepted limits (>100 MPN/mL) in all sites. In addition, results showed that the concentration levels of *E. faecalis* were higher in sediments than in water. In addition, the isolated strains were highly resistant to some antibiotics such as rifampicin, gentamicin, chloramphenicol, ampicillin and azithromycin. The results of this study confirmed the alarming situation caused by bacterial input loads from wastewater discharges, particularly with the isolation of multidrug resistance strains.

Table 1 | Bacterial analysis of seawater and sediment samples from Tunisian coast

Parameters sites	Samples	Fecal coliforms	Total coliforms	<i>Enterococcus faecalis</i> (MPN/100 mL)	<i>Salmonella</i> (MPN/1,000 mL)	<i>Vibrio cholerae</i> (MPN/1,000 mL)	Cultivable heterotrophic bacteria (CHB)	Vibrionaceae thermotolerant (VB)	References
Rejiche	Seawater	<3 (MPN/100 mL)	–	<3–> 2,400 (MPN/100 mL)	0	0	–	–	Alibi <i>et al.</i> (2021)
Rejiche	Sediment	<3 (MPN/100 mL)	–	93–> 2,400 (MPN/100 mL)	0	0	–	–	Alibi <i>et al.</i> (2021)
Monastir	Seawater	0	–	–	–	–	–	–	Zaafrane <i>et al.</i> (2022)
Bizerte lagoon	Seawater	–	1–2 × 10 ³ ufc/100 mL	1–10 ³ ufc/100 mL	–	–	–	–	Boukef <i>et al.</i> (2008)
	Sediment	–	<10–10 ⁷ ufc/100 mL	–	–	–	–	–	Boukef <i>et al.</i> (2008)
	Seawater	–	–	–	–	–	10 ⁴ –10 ⁸ ufc/100 mL	50–10 ⁵ ufc/100 mL	Boukef <i>et al.</i> (2010)
	Sediment	–	–	–	–	–	10 ⁸ –10 ⁹ ufc/100 mL	10 ⁴ and 10 ⁶ ufc/100 mL	Boukef <i>et al.</i> (2010)
Bizerte lagoon	Seawater	–	1–3 (ULog)	–	–	–	–	–	Boukef <i>et al.</i> (2012)
Bizerte lagoon	Sediment	–	<1–6 (ULog)	–	–	–	–	–	Boukef <i>et al.</i> (2012)

Previous studies investigated in the Bizerte lagoon (Table 1) have shown that coliform contamination is higher in sediments rather than in the water column and increased during the summer season. In addition, sediment and seawater samples from the same locality showed the presence of *Vibrio* spp. according to a study conducted by Gdoura *et al.* (2016). The same study showed the presence of *V. parahaemolyticus* in both seawater (10%) and sediment samples (30%) from different localities in Sfax, Djerba and Gabes. Nevertheless, *V. alginolyticus* (12.5%) was only found in sediment samples.

Hassen *et al.* (2022) investigated the microbiological quality of wastewater released into the Mediterranean Sea. Wastewater sampling was carried out in Tunisia and other Mediterranean nations. (Egypt, Morocco, Algeria and Italy). The quality of the treated wastewater and seawater appeared to be adequate and satisfied the standard and recommended limits in vigor in Tunisia and others countries. The bacteriological quality of these waters and their diversification was assessed by the denaturing gradient gel electrophoresis (DGGE) technique. The examination of antibiotic susceptibility at the level of 18 strains isolated from various arbitrary water samples showed that most of these isolates exhibited resistance to at least one antibiotic family, and 11 isolates exhibited 100% resistance against aztreonam. These results confirm the large antibiotic-resistance spreading and circulation registered these last decades in the natural environment, conditioned by the extensive and non-controlled use of antibiotics for human therapy and animal feeding.

Bacterial loads were also detected in marine organisms such as bivalve mollusks known for their accumulation of microorganisms. Table 2 illustrates the bacterial species found in Tunisian bivalve mollusks and fish during the last years.

Clams (*Ruditapes decussatus*) gathered from the Sfax coast in southern Tunisia were tested for contamination levels by *Escherichia coli* and *Salmonella* spp. The results revealed that 59 samples of *R. decussatus* out of the 539 analyzed were contaminated with *Salmonella* spp. (11%) and 194 samples (36%) had levels of *E. coli* contamination that were above the regulatory limit (>230 CFU/100 g FIL). Besides, *E. coli* contamination rose significantly between the summer and winter periods (21–23%) that is explained by the presence of migratory birds, significant tourist activity and variation in temperature. A total of 28 of the 59 cases (47.4%) had high levels of *E. coli* and *Salmonella* spp. contamination during two periods of the year: from March to May and from September to November over a study period of 4 years (2013–2016) (Zormati *et al.* 2018).

Table 2 | Bacteria species detected in the edible bivalve and fish species distributed in the Tunisian coastlines

Microorganisms	Host	Prevalence	Site	Sources
<i>Salmonella</i> spp.	<i>Mytillus galloprovincialis</i> / <i>Ruditapes decussatus</i> /oyster	0%	Tunis/Bizerte/Gabes	Amri <i>et al.</i> (2011)
<i>Salmonella</i> spp.	<i>Ruditapes decussatus</i>	11%	Sfax coast	Zormati <i>et al.</i> (2018)
<i>E. coli</i>	<i>Mytillus galloprovincialis</i> / <i>Ruditapes decussatus</i> /oyster	57% (>230 CFU/100 g)	Tunis/Bizerte/Gabes	Amri <i>et al.</i> (2011)
<i>E. coli</i>	<i>Ruditapes decussatus</i>	36% (>230 CFU/100 g)	Sfax coast	Zormati <i>et al.</i> (2018)
<i>Vibrio alginolyticus</i>	<i>Mytilus edulis</i> <i>Crassostrea gigas</i>	20 strains	Bizerte lagoon	Snoussi <i>et al.</i> (2010)
<i>Vibrio alginolyticus</i>	<i>Ruditapes decussatus</i>	85.7%	Sfax/Djerba/Gabes/ Bizerte	Gdoura <i>et al.</i> (2016)
<i>Vibrio alginolyticus</i>	<i>Sparus aurata</i>		Sousse/Cheba	Zouiten <i>et al.</i> (2017)
<i>Vibrio alginolyticus</i>	Fish	74.6%	Sfax/Djerba/Gabes/ Bizerte	Gdoura <i>et al.</i> (2016)
<i>Vibrio fluvialis</i>	<i>Crassostrea gigas</i>	1 strain	Bizerte lagoon	Snoussi <i>et al.</i> (2010)
<i>Vibrio parahaemolyticus</i>	<i>Crassostrea gigas</i>	1 strain	Bizerte lagoon	Snoussi <i>et al.</i> (2010)
<i>Vibrio parahaemolyticus</i>	<i>Ruditapes decussatus</i>	14.3%	Sfax/Djerba/Gabes/ Bizerte	Gdoura <i>et al.</i> (2016)
<i>Vibrio parahaemolyticus</i>	<i>Dicentrarchus labrax</i>	9%	Sousse	Khoudja <i>et al.</i> (2013)
<i>Vibrio parahaemolyticus</i>	<i>Sparus aurata</i>	9%	Sousse	Khoudja <i>et al.</i> (2013)
<i>Vibrio parahaemolyticus</i>	<i>Crassostrea gigas</i>	10%	Lac of Bizerte	Khoudja <i>et al.</i> (2013)
<i>Vibrio parahaemolyticus</i>	<i>Mytilus edulis</i>	5%	Lac of Bizerte	Khoudja <i>et al.</i> (2013)
<i>Vibrio parahaemolyticus</i>	<i>Sparus aurata</i>	100%	Monastir/Cheba/ Sousse	Zouiten <i>et al.</i> (2017)
<i>Vibrio parahaemolyticus</i>	Fish	3.7%	Sfax/Djerba/Gabes/ Bizerte	Gdoura <i>et al.</i> (2016)
Total Vibrionaceae	<i>Lithophaga lithophaga</i>	$6 \times 10^6 - 8 \times 10^6$ CFU mL ⁻¹	Bizerte Bay	Jaafar <i>et al.</i> (2012)
<i>Vibrio</i> spp.	Fish	43.4%	Sfax/Djerba/Gabes/ Bizerte	Gdoura <i>et al.</i> (2016)
<i>Vibrio cholerae</i>	Fish	1.7%		Gdoura <i>et al.</i> (2016)

Amri *et al.* (2011) studied the bacteriological contamination of shellfish collected between 2007 and 2008 from the north (Lake of Tunis, the canal of Tunis, Menzel Jemil, Bizerte) and the south (Gabes) of Tunisia. The results obtained showed that none of the samples revealed the presence of *Salmonella*. However, from 54 samples analyzed, they found that 31 (57%) samples showed a number of *E. coli* over the European legislation.

The genus *Vibrio* including more than 100 species is widely distributed in aquatic and marine habitats. A large number of *Vibrio* species are associated with marine organisms like fish, mollusks and crustaceans in commensal or pathogenic relations (Romalde *et al.* 2014). Various species of the genus *Vibrio* constitute a major threat to public health; and *Vibrio* bacteria are most often the causative agents of disease and death related to the consumption of clams and fish.

Several studies describing the presence of *Vibrio* spp. in seawater, sediment and a variety of seafood include shrimp, crab, oysters and clams, due to its halophilic characteristics.

In a study conducted by Bakr *et al.* (2011), *Vibrio* was isolated from 52% of tested seafood's (shrimp, oysters and mussel) from different markets in Alexandria (Egypt) with the highest percentage (88%) from oysters. The most frequently isolated *Vibrio* spp. were *V. alginolyticus* (52.5%), *V. parahaemolyticus* (14.1%) and *V. mimicus* (11.5%). A similar study carried out by Elal Mus *et al.* (2014) examined seafood samples including fish, mussel and shrimp from Bursa markets (Turkey). The results revealed that 67% were found to be contaminated with *Vibrio* spp. such as *V. parahaemolyticus* (28%), *V. vulnificus* (1%) and *V. cholerae* (1%). Mannas *et al.* (2014) reported the occurrence of different *Vibrio* species in *Mytilus galloprovincialis* samples collected along the Moroccan Atlantic coast with the predominant isolated *Vibri*os were *Vibrio alginolyticus* (90.4%), followed by *V. cholerae* (15.4%) and *V. parahaemolyticus*.

In Tunisia, numerous studies (Snoussi *et al.* 2008; Khouadja *et al.* 2013; Zrelli *et al.* 2015; Gdoura *et al.* 2016; Zaafrane *et al.* 2022) conducted in different coastal sites reported the presence of *Vibrio* spp. in seawater, sediment and marine organisms.

Snoussi *et al.* (2008) reported that seawater of Monastir seacoast harbors different genotypes of *V. alginolyticus* strains that inherited several virulence genes from autochthonous bacteria such as *V. cholera* able to produce several virulence enzymes and exhibit a high power to adhere to human epithelial cells and fish mucus. Other bacteria belonging to *Vibrio* and *Aeromonas* genus were identified with low frequency such as: *Vibrio parahaemolyticus*, *Vibrio vulnificus*, *Vibrio fluvialis*, *Vibrio hollisae* and *Aeromonas hydrophila*. Further study conducted by the same author (Snoussi *et al.* 2010) reported the isolation of *Vibrio* species from mussels and oysters in mollusk farm located in Bizerte lagoon.

A study carried out by Zrelli *et al.* (2015) examined the prevalence of *V. parahaemolyticus* for 70 samples of purified wild clams collected randomly from markets and originated from Sfax and Gabes production zone during the harvesting season (October 2012 to May 2013). The research was performed according to the standard method ISO/TS 21872-1:2007 and PCR protocol was used for confirmation of *V. parahaemolyticus* and for the detection of virulence genes (tdh and trh). The results showed a very low prevalence (1.4% or 1/70). The strain of *V. parahaemolyticus* isolated was a carrier of the virulence gene tdh and is potentially pathogenic. This study confirmed that the risk associated with *Vibrio* in marine bivalve is not completely controlled by purification, hence the importance of combining this process with other preventive measures such as respecting the cold chain and cooking.

In total, 247 samples, including 113 fishes, 83 clams (*Ruditapes decussatus*), 30 seawater samples and 21 sediment samples were collected from different localities in Sfax, Djerba, Gabes and Bizerte from August to October 2012 and from July to November 2014. These samples were analyzed for *Vibrio* spp. identification using traditional culture methods and a conventional PCR method. The results showed that the conventional culture method found 102 (41.3%) of 247 analyzed samples positive for *Vibrio* spp.; a conventional PCR method found 126 (51%) of the 247 samples positive. Real-time PCR assay found 126 (51.1%) samples positive; *V. alginolyticus* toxR was the most common, found in 99 (78.57%) of samples, followed by *V. parahaemolyticus* in 26 (20.63%) and *V. cholerae* in 1 (0.7%) (Gdoura *et al.* 2016).

Further studies have been conducted by Zaafrane *et al.* (2022) in order to investigate the presence of *Vibrio* species and especially *V. parahaemolyticus* in 66 seawater samples collected during 2018 from 15 stations from the north to the south along the Tunisian coast. The results showed the detection of *Vibrio alginolyticus* in all samples. However, both *Vibrio cholerae* and *Vibrio vulnificus* were not found. Besides, only eight samples containing *V. parahaemolyticus* were detected.

The rare presence of *V. parahaemolyticus* in Tunisian coastal seawaters was also proved in previous studies (Snoussi *et al.* 2008; Khouadja *et al.* 2013; Zrelli *et al.* 2015; Gdoura *et al.* 2016). This low occurrence of *V. parahaemolyticus* in Tunisian coasts compared to investigation results found in other Mediterranean countries (Caburlotto *et al.* 2016) is probably attributed to poor adaptation of this bacterium to southern Mediterranean conditions, which is characterized by high temperature, sunlight intensity and important salinities. Zouiten *et al.* (2017) reported the presence of *Vibrio parahaemolyticus* and *Vibrio alginolyticus* strains in wild and farm sea bream (*Sparus aurata*) in the central coast of Tunisia (Chebba, Sousse, Monastir). This study showed a difference between farmed fish and wild fish resistance to amoxicillin antibiotics explained by the excessive use of antibiotics colistin and fucidic acid during treatment in farm fish.

3. VIRAL CONTAMINATION

Viruses are the most abundant members of marine microbial communities. Enteric viruses represent a diverse group of pathogens that are responsible for a variety of human diseases, from ocular and respiratory infections to gastroenteritis, hepatitis, myocarditis and aseptic meningitis (Gonçalves *et al.* 2018). These pathogens are excreted in the feces of infected individuals

and are primarily transmitted by the fecal–oral route. They are introduced into the marine environment mainly through the discharge of treated and untreated wastes. These viruses have high stability and persistence they can survive in a marine environment for long periods and can be carried for long distances, even several kilometers from the point of discharge (Cioffi *et al.* 2020). RoV and NoV are among the most commonly present enteric viruses in polluted coastal waters and the main agents of viral gastroenteritis worldwide (Girones *et al.* 2010; Lin & Ganesh 2013; Robilotti *et al.* 2015).

The virus present in seawater and sediment can be transmitted to marine organisms such as bivalve mollusks. Virus uptake by shellfish is a fast and dynamic process and high titers of virus can be accumulated by shellfish in a short period of time. Shellfish have been identified as a vector for human enteric pathogens for more than 150 years (Le Guayder *et al.* 2006).

The presence of viral pathogens in edible mollusks is shown in several studies. In Tunisia, enteric viruses have been detected in bivalve mollusks grown along the Tunisian coastline. Table 3 lists the virus species detected in bivalve mollusks from Tunisian coasts.

Since 2006, Elamri *et al.* (2006) have revealed viral contamination of shellfish (*Mytillus galloprovincialis*, *Ruditapes decussatus*) collected from northern Tunisia between 2000 and 2001. Overall, 83% of the samples were found positive for at least one virus. AsV was detected in 61% of the samples, NoV in 35%, HAV in 26% and enterovirus in 4.3% (one mussel).

A similar study conducted by Gharbi-Khelifi *et al.* (2007) showed the detection of HAV in five (20%) samples of *Ruditapes decussatus* flesh collected at Monastir and Sfax sites. However, the virus was not detected in samples from the Bizerte site situated in northern Tunisia and characterized by different climatic conditions (high rainfall, low temperature) (Yazidi *et al.* 2017).

Subsequently, two separate studies (Sdiri-Loulizi *et al.* 2010a, 2010b) revealed that 1.6 and 6.6% of shellfish collected between 2003 and 2007 from the Monastir coast near a wastewater outlet site, tested positive for NoV (GI.2) and Aichi virus (AiV A), respectively.

Additional study has been conducted by Amri *et al.* (2011) for shellfish (*Mytilus galloprovincialis*, *Ruditapes decussatus*, *Crassostrea gigas*) collected between 2007 and 2008 from the north (Lake of Tunis, the canal of Tunis, Menzel Jemil, Bizerte) and south (Gabes) of Tunisia. Overall, the HAV was detected in 32% of the shellfish samples analyzed. The detection of HAV

Table 3 | Virus species detected in the edible bivalve species distributed in the Tunisian coastlines

Microorganisms	Host	Prevalence (%)	Site	Sources
Astrovirus	<i>Mytillus galloprovincialis</i> / <i>Ruditapes decussatus</i>	61	North coast	Elamri <i>et al.</i> (2006)
Norovirus	<i>Mytillus galloprovincialis</i> / <i>Ruditapes decussatus</i>	35	North coast	Elamri <i>et al.</i> (2006)
Norovirus	<i>Ruditapes decussatus</i>	19	Sfax coast	Zormati <i>et al.</i> (2018)
Norovirus	Shellfish samples	1.6	Monastir Bay	Sdiri-Loulizi <i>et al.</i> (2010a)
Hepatitis A (HAV)	<i>Mytillus galloprovincialis</i> / <i>Ruditapes decussatus</i>	26	North coast	Elamri <i>et al.</i> (2006)
Hepatitis A (HAV)	<i>Mytillus galloprovincialis</i> / <i>Ruditapes decussatus</i> / <i>Crassostrea gigas</i>	62	Lac de Tunis	Amri <i>et al.</i> (2011)
Hepatitis A (HAV)	<i>Mytillus galloprovincialis</i> / <i>Ruditapes decussatus</i> / <i>Crassostrea gigas</i>	50	Canal de Tunis	Amri <i>et al.</i> (2011)
Hepatitis A (HAV)	<i>Mytillus galloprovincialis</i> / <i>Ruditapes decussatus</i> / <i>Crassostrea gigas</i>	13	Menzel Jemil	Amri <i>et al.</i> (2011)
Hepatitis A (HAV)	<i>Mytillus galloprovincialis</i> / <i>Ruditapes decussatus</i> / <i>Crassostrea gigas</i>	25	Gabes	Amri <i>et al.</i> (2011)
Hepatitis A (HAV)	<i>Ruditapes decussatus</i>	20	Monastir and Sfax coast	Gharbi-khelifi <i>et al.</i> (2007)
Hepatitis A (HAV)	<i>Ruditapes decussatus</i>	3	Sfax coast	Zormati <i>et al.</i> (2018)
Aichivirus	Shellfish samples	6.6	Monastir Bay	Sdiri-Loulizi <i>et al.</i> (2010b)
Enterovirus	<i>Mytillus galloprovincialis</i> / <i>Ruditapes decussatus</i>	4.3	North coast	Elamri <i>et al.</i> (2006)

in these samples revealed that the contamination rate is higher in samples from the lake and the canal of Tunis; 62 and 50%, respectively. The Bizerte production area showed a lower rate (37%) followed by the Gabes zone (25%). The lowest contamination rate is noted for samples from Menzel Jemil (13%). Furthermore, clams were found more contaminated with HAV than mussels and oysters probably because of their burrowing way of life. While the presence of the HAV virus was confirmed in fish and shrimp samples caught from the Persian Gulf in Iran (Alipour Amroabadi *et al.* 2021), this virus was detected only in bivalves in Tunisia.

A similar study was carried out in the Sfax region (Zormati *et al.* 2018) during which microbiological contamination risk factors for clams (*Ruditapes decussatus*) were examined over four consecutive years (2013–2016). A total of 252 samples of live clams were analyzed for contamination by HAV and NoV in different clam production areas. The prevalence of positive samples was 19% for NoV and 3% for HAV.

4. PARASITOLOGICAL CONTAMINATION

Parasites are natural components of ecological systems, as well as agents of the outbreak and disease-induced mortality (Marcogliese 2002). Nowadays, Coastal ecosystems are threatened by the improper disposal of sewage, illegal wastewater discharge and artificial and natural runoff. In fact, feces from humans, their pets, and domesticated and wild animals may carry numerous pathogenic microorganisms, which can easily contaminate the sea and its inhabitants when released into estuaries and marine environments worldwide (Ghozzi *et al.* 2017).

No national protozoa-monitoring program exists in estuaries and coastal waters despite the presence of these parasites in marine waters and in the food chain.

4.1. Protozoans of human and zoonotic interest

Protozoan of human and zoonotic parasites are transmitted to the marine environment and can easily contaminate the sea and its inhabitants (Giangaspero *et al.* 2014). Over the last two decades, these protozoa have been found to enter aquatic environments and contaminate shellfish, which appears as a new potential risk of fish-borne protozoan infections (Moratal *et al.* 2020). Among these parasites, *Cryptosporidium*, *Giardia* and *Toxoplasma gondii*, are important pathogens that are increasingly found in the marine environment worldwide.

They are transmitted through oocysts and cysts, which are excreted in high quantities in the infected hosts' feces. Marine organisms (bivalve, mammals) are susceptible to infection with the zoonotic protozoan parasites *Giardia*, *Cryptosporidium* and *Toxoplasma* (Herder *et al.* 2015; Reboredo-Fernández *et al.* 2015; Van de Velde *et al.* 2016) and are increasingly exposed to these pathogens due to runoff, farming and anthropogenic impacts in coastal areas (VanWormer *et al.* 2016).

Several studies indicate that these encysted protozoan pathogens are also in coastal waters, having contaminated a variety of shellfish and infected many species of marine mammals. These findings raise concern for the health of animals in coastal waters and for humans who eat raw shellfish and who use these waters for recreation (Fayer 2004). In recent times, the apicomplexan *Toxoplasma gondii* has been diagnosed frequently in several species of fish and cetaceans stranded along Italian and Spanish Mediterranean coasts (Bernal-Guadarrama *et al.* 2014; Di Guardo & Mazzariol 2016; Marino *et al.* 2019). In addition, several edible and inedible shellfish have been found to carry protozoan pathogens, either alone and/or in association (Ghozzi *et al.* 2017).

The role of marine bivalve mollusks as disseminators of protozoan parasites to humans and animals have been addressed in several studies around the world (Aksoy *et al.* 2014; Palos Ladeiro *et al.* 2014; Tedde *et al.* 2019; Nayeri *et al.* 2021) because these mollusks are filter feeders that are able to retain oocysts and cysts of various protozoa in their tissue. Zoonotic protozoan parasites; *Giardia*, *Cryptosporidium* and *Toxoplasma* are increasingly found in the most consumed marine mollusk species worldwide such as clams, mussels and oysters. They retain their infectivity in raw or undercooked mollusk.

According to Erickson & Ortega (2006) *Cryptosporidium* oocysts entering marine waters may survive for 1 year in seawater at 6–8 °C; however, an increasing salinity is deleterious to the parasites. Indeed, Fayer *et al.* (1998) showed that oocysts suspended in water at a salinity of 20 and 30 ppt at 20 °C retain their infectivity for 8 and 4 weeks, respectively. While *Toxoplasma* oocysts can sporulate in seawater at salinities of 15 and 32 ppt at 24 °C (Lindsay *et al.* 2003). Sporulated *Toxoplasma* oocysts retain their infectivity for at least 6 months in seawater at 15 ppt at the same temperatures. This parasite acts as an indicator of marine pollution and marine mammal health (Hohwey *et al.* 2013). *Giardia* cysts can survive for long periods in the environment under cool, moist conditions. In various experiments, cysts were shown to survive in seawater for more than 2 months at 4 °C (Erickson & Ortega 2006). In fact, the oocyst walls of these parasites are the key structures that

confer great resistance to various conditions and inactivation procedures and secure the transport of the oocysts from the environment to the host.

Monitoring occurrences of zoonotic protozoa in the marine environment is important for sanitary goals and may serve as bioindicators of contamination. In the last 15 years, *Giardia*, *Toxoplasma* and *Cryptosporidium* parasites have been detected on many occasions in naturally contaminated mollusks from different areas worldwide (Coupe *et al.* 2018; Cong *et al.* 2019, 2021; Marquis *et al.* 2019; Tedde *et al.* 2019).

In Tunisia, only one study conducted by Ghazzi *et al.* (2017) investigated for the first time the contamination by protozoan parasites along the Tunisian coasts. Biological contamination with pathogenic protozoa has been studied for four intestinal protozoan parasites *Giardia duodenalis*, *Cryptosporidium*, *Toxoplasma gondii* and *Cyclospora cayetanensis* using wild shellfish collected along the Tunisian coastline as environmental biosentinels. Protozoans were detected in 6.9% of the investigated marine bivalve mollusks, two of the nine investigated coastal sites harbored protozoan parasites; one in the central coast (Monastir Bay – Khnis) and one in the south (Gulf of Gabès-Jaboussa).

These results provide the first evidence that Tunisian coastal waters are contaminated by zoonotic protozoan parasites and can constitute a direct (through bathing or recreational activities on the beaches or inflowing watercourses) or indirect (through the consumption of raw or undercooked bivalves) risk for human health.

4.2. Protozoans of sanitary interest for shellfish

Protozoan parasites are common pathogens of many marine bivalve mollusks. They represent valuable markers of animal and ecosystem health and reflect food web dynamics by acting as indicators for diet and trophic interactions (Marcogliese 2002). Parasitic organisms show enormous biological diversity, reflecting adaptations to the parasitic way of life in different types of hosts and in diverse sites and environments.

In the marine environment, parasites and diseases are currently a challenge to bivalve aquaculture practices. Their control relies on biosecurity measures to prevent their introduction and limit their spread (Fernández-Boo *et al.* 2014, 2021).

Protozoans of the genera *Perkinsus*, *Marteilia* and *Bonamia* are currently recognized as major threats to natural and farmed bivalve populations around the world. They can mainly infect oyster and clam species, causing enormous damage to commercial productions and they are currently under surveillance by World Organization for Animal Health (OIE) (Fernández Robledo *et al.* 2014, Zannella *et al.* 2017). These parasites have been shown to reduce fecundity, alter burrowing behavior and limit growth (Longshaw & Malham 2013). Experimental studies have demonstrated infective stages for many of these parasites, including *Perkinsus marinus*, *Perkinsus olseni*, *Marteilia sydneyi* and *Bonamia ostreae* can survive for days, weeks and even months in the water column, and remain infective when they enter a new host (Ben-horin *et al.* 2015).

Marteilia refringens is a Paramyxia protozoan parasite infecting the digestive system of commercially important bivalve species (oyster *Ostrea edulis*, the mussels *Mytilus edulis* and *Mytilus galloprovincialis*) and inducing physiological disorders and eventually death of the animal (Elgharsalli *et al.* 2013). It has been recorded in Europe from the northern French coast southwards to the Mediterranean Sea, plus Corsica, Italy (including Sardinia), Slovenia, Portugal, Croatia, Greece and Tunisia (Kerr *et al.* 2018).

The genus *Perkinsus* includes parasites that infect mollusks worldwide, some of which are associated with serious diseases and mass mortalities (Corbeil & Berthe 2009). Among *Perkinsus* spp., *P. marinus* and *P. olseni* are the most destructive species. *Perkinsus olseni* has been detected infecting several clam and oyster species from Australia, New Zealand, South Korea, Japan, China, Portugal, Spain and Italy.

Bonamia species are haplosporidian protozoan parasites infecting the haemocytes of several oyster species. Three species of *Bonamia* have been identified worldwide, *B. exitiosa* from the Mediterranean Sea, America, Australia and New Zealand; *B. ostreae* in Europe and North America and *B. perspora* from North America. Most of the microscopic signs of *Bonamia* spp. infection is the haemocytic infiltration in all the connective tissue (Vázquez & Cremonte 2017).

In Tunisia, some studies proved the presence of such protozoan parasites in some oyster and clam species. Hill *et al.* (2010) evaluated the *Bonamia* spp. infection status of the oyster *Ostrea stentina* from Hammamet coast, collected in June 2007. The examination of 85 *Ostrea stentina* revealed nine oysters (10.6%) PCR- positive for a *Bonamia* sp. using a *Bonamia* genus-specific assay. Table 4 shows parasite species detected in Tunisian bivalve mollusks.

Another study conducted by El Bour *et al.* (2012) has detected perkinsosis in carpet shell clam populations (*Ruditapes decussatus*) in three sites in the Tunisian lagoon and the North Lake of Tunis. The results showed that perkinsosis was

Table 4 | Parasites species detected in bivalve species distributed in Tunisian coastlines

Microorganisms	Host	Prevalence (%)	Site	Sources
<i>Bonamia</i> spp.	<i>Ostrea stentina</i>	10.6	Hammamet	Hill <i>et al.</i> (2010)
<i>Marteilia refringens</i>	<i>Ostrea stentina</i>	88	Monastir Bay	Elgharsalli <i>et al.</i> (2013)
<i>Bonamia exitiosa</i>	<i>Ostrea stentina</i>	2.91	Monastir Bay	Elgharsalli <i>et al.</i> (2016)
<i>Perkinsus olseni</i>	<i>Ruditapes decussatus</i>	2–60	North Lake of Tunis	El Bour <i>et al.</i> (2012)
<i>Giardia duodenalis</i>	<i>Ruditapes decussatus</i>	1.6	Monastir Bay	Ghozzi <i>et al.</i> (2017)
<i>Toxoplasma gondii</i>	<i>Ruditapes decussatus</i>	6.6	Monastir Bay	Ghozzi <i>et al.</i> (2017)
<i>Cyclospora cayetensis</i>	<i>Ruditapes decussatus</i>	1.6	Monastir Bay	Ghozzi <i>et al.</i> (2017)

detected preferentially in winter periods from the external and marine sites with the prevalence varying from 2 to 60%. Whereas, in summer periods the frequency of perkinsosis was lower and only detected at internal sites of North Lake, with rates ranging from 2 to 8%.

After reports of massive mortality of the European flat oyster *Ostrea stentina* along the Tunisian coasts, Elgharsalli *et al.* (2013) have identified for the first time, the parasite *Marteilia refringens* using a *Marteilia* genus-specific assay in samples of this oyster collected from Monastir Bay coast. This study revealed the presence of *M. refringens* DNA in 88% of the oysters *Ostrea stentina*. Histological and transmission electron microscopy analyses confirmed the presence of this parasite in the digestive gland tissue of this species and characterized it at the ultrastructural level. These results suggested a possible involvement of *M. refringens* in the mortality of *O. stentina* in Tunisian coasts.

In a further recent study by the same author (Elgharsalli *et al.* 2016), the prevalence of bonamiosis and marteiliosis in the dwarf oyster *Ostrea stentina* were established by PCR. The results showed that *Bonamia exitiosa* was detected only in 2.91% of oysters while the prevalence of *M. refringens* was between 100 and 93.93%. During the study period, a 42–87% mortality rate was reported.

5. DISEASES CAUSED BY PATHOGENS FOUND IN MARINE ENVIRONMENT

Waterborne pathogens, including bacteria, viruses and protozoa, are a direct threat to human and animal health. Among those, pathogens transmitted to the coastal area can cause a wide range of diseases and symptoms such as gastroenteritis, ocular, respiratory infections, hepatitis, myocarditis and life-threatening infections in immunocompromised people. Table 5 summarizes the principles' pathogens transmitted to coastal areas their associated diseases and their occurrence in the Tunisian marine environment.

The majority of commonly occurring waterborne pathogens are linked to fecal sources on land. They spread to humans through ingestion of contaminated water, exposure to contaminated water from recreational activities like swimming or indirectly through contaminated seafood.

The transport and the fate of waterborne pathogens into the coastal areas is influenced by several factors such as precipitation and runoff; the type and location of sources on land; and the survival characteristics of microorganisms (Malham *et al.* 2014).

Among bacteria pathogens, *Salmonella* is widely distributed in coastal water. This microorganism is the principal cause of salmonellosis, which is one of the most prevalent zoonotic diseases in the world (Antilles *et al.* 2021). Moreover, *Escherichia coli* is one of the species implied in the outbreaks of infantile clinical pathology (Boukef Ben Omrane *et al.* 2011). *Vibrio* genus is known as bacteria that exist mainly in the coastal regions or mouth of rivers. Some types cause infections in humans, including *Vibrio cholerae*, *Vibrio vulnificus*, *Vibrio parahaemolyticus* and *Vibrio alginolyticus*. Waterborne diseases caused by *Vibrio* species are often known to cause gastritis when raw or undercooked seafood is eaten (Jung 2018). Halophilic *Vibrio* species are known to cause mild infections in humans, but can also cause high morbidity, mortality or infections in fish and other aquatic animals. In many epizootic events, *Vibrio alginolyticus* is often isolated from diseased fish cultured in Tunisia and is associated with mass mortality (Ben Kahla-Nakbi *et al.* 2006, 2007). The same author confirmed that the pathogenicity of *V. alginolyticus* may be the result of a combination of many factors including the ability to produce extracellular products (ECPs), growth in iron-limiting conditions, survival in fish serum and the presence of *V. cholerae* virulence genes in the *V. alginolyticus* genome (Ben Kahla-Nakbi *et al.* 2009).

Table 5 | Pathogens transmitted to coastal areas, their associated diseases and their occurrence in Tunisian marine environment; adapted from Griffin *et al.* (2003), Malham *et al.* (2014), Saxena *et al.* (2015)

Pathogens	Diseases	Symptoms/illness	Occurrence in Tunisian coastal water (W), sediment (Sd), shellfish (S) or fish (F)
Bacteria			
<i>Campylobacter jejuni</i>	Gastroenteritis	Diarrhea, dysentery, fever, severe abdominal cramps	
<i>Enteropathogenic E. coli</i>	Gastroenteritis	Vomiting, diarrhea	S
<i>Salmonella</i>	Typhoid, paratyphoid, salmonellosis	Diarrhea, dehydration, high fever, ulceration of the small intestine	S
<i>Shigella</i>	Shigellosis	Fever, diarrhea (often bloody), abdominal pain, stomach cramps	
<i>Legionella</i>	Legionellosis	Pneumonia-type illness, mild flu-like illness	
<i>Vibrio vulnificus</i>	Gastroenteritis	Diarrhea, vomiting, abdominal pain	S
<i>Vibrio cholerae</i>	Cholera	Vomiting, watery diarrhea, abdominal pain, dehydration	F
<i>Vibrio parahaemolyticus</i>	Gastroenteritis	Diarrhea, nausea, vomiting, chills, headache, abdominal pain	W, S, F, Sd
Viruses			
Calicivirus	Gastroenteritis	Vomiting, diarrhea	
Adenovirus	Gastroenteritis	Respiratory illness, eye infection, diarrhea, vomiting	
Enterovirus		Fever, headache, respiratory illness and sore throat	S
Norovirus	Gastroenteritis	Nausea, vomiting, diarrhea, stomach pain	S
Aichivirus	Gastroenteritis	Diarrhea, abdominal pain, nausea, vomiting and fever	S
Astrovirus	Gastroenteritis	Diarrhea, vomiting	S
Rotaviruses	Gastroenteritis	Infantile diarrhea, vomiting	
Echovirus		Fever, meningitis, diarrhea, respiratory illness	
Polioviruses		Meningitis, paralysis	
Hepatitis A	Infectious hepatitis	Fever, nausea, lassitude, abdominal pain, jaundice	S
Norwalk viruses	Gastroenteritis	Diarrhea, vomiting, fever	
Coxsackievirus		Meningitis, respiratory illness, myocarditis	
Protozoans			
<i>Cryptosporidium</i>	Cryptosporidiosis	Diarrhea, nausea, weight loss, low-grade fever	
<i>Entamoeba histolytica</i>	Amoebic dysentery	Stomach pain, bloody stools, fever	
<i>Giardia lamblia</i>	Giardiasis	Diarrhea, nausea, indigestion, weight loss	S
<i>Toxoplasma gondii</i>	Toxoplasmosis	^a Fever, muscle aches, sore throat, ocular toxoplasmosis	S
<i>Cyclospora cayatanensis</i>	Cyclosporiasis	Diarrhea, weight loss, stomach cramps, bloating and farting, muscle aches	S

^aPregnant women and immunocompromised people.

Several outbreaks of viral food-borne diseases have been reported due to the consumption of seafood harvested from sewage-contaminated waters (Hall *et al.* 2014; Parrón *et al.* 2019). Of over 100 virus species which cause a wide variety of illnesses in humans, and only a few of these viral pathogens have been shown epidemiologically to be waterborne. Among those, NoV, AsV, RoV, AdV as well as hepatitis A, E virus are health significant viruses that may be acquired by ingestion of contaminated water or shellfish. The exact risk of illness from enteric viruses in water after exposure is difficult to quantify, as asymptomatic infections are particularly common in some enteric viruses. The development of clinical illness depends on numerous factors including the immune status and age of the host, type, strain and virulence of the microorganism (Bosch 1998).

Furthermore, waterborne parasitic protozoan outbreaks are widely reported in both developed and developing countries. The most prevalent waterborne parasitic infections are cryptosporidiosis and giardiasis. Other parasitic protozoa with waterborne transmission that cause human infections are *Toxoplasma gondii* and *Cyclospora cayetanensis*.

The highest prevalence of waterborne protozoan outbreaks was reported in developed countries. According to the study carried by Ma *et al.* (2022) during the period from 2017 to 2020, out of 251 outbreaks were reported worldwide. Most protozoan outbreaks were associated with *Cryptosporidium* (192 outbreaks) than *Giardia* (48 outbreaks), *Toxoplasma gondii* (seven outbreaks) and *Cyclospora cayetanensis* (seven outbreaks). The highest prevalence of waterborne protozoan outbreaks was reported in developed countries.

Toxoplasmosis represents a high risk for pregnant women and immunocompromised patients by developing the clinical disease with harsh outcomes, including congenital toxoplasmosis and life-threatening encephalitis.

Worldwide, *T. gondii* is known to cause sporadic clinical disease and fatality in marine mammals. During the last decades, a number of studies have reported *T. gondii* infection in marine animals (sea otters, cetaceans, pinnipeds, sirenian) with the highest prevalence recorded in the population of sea otters (54.8%). It has been reported that *T. gondii* encephalitis in sea otters causes a high mortality rate and is responsible for slow population recovery (Ahmadpour *et al.* 2022).

6. DISCUSSION AND CONCLUSION

This review has covered microbial contamination cases in Tunisian coasts over different studies conducted in seawater, sediment and marine organisms. Numerous studies have reported the presence of microorganism's pathogens on Tunisian coasts. The monitoring for fecal pollution using fecal indicator bacteria showed the effect of wastewater discharges in the coastal areas, which increases the bacterial charge in both water and sediment. Although a recent study showed the absence of fecal pollution in some coastal areas like Monastir Bay (Zaafraane *et al.* 2022), fecal indicators such as *Escherichia coli*, *Salmonella* spp. were detected and were in some cases above the regulatory limit in samples of clams and mussels. Several environmental factors affect microbial contamination levels in marine environments such as the season (Temperature, irradiation), tourism and the presence of migratory birds. These birds gather by the seashore to feed and can carry in their feces pathogens contaminating both the water and marine food product (Zormati *et al.* 2018). In fact, these pathogens are filtered and accumulated by shellfish consumed by humans and marine mammals and can infect a wide range of marine animal hosts.

On the other hand, the presence of Vibrionaceae species was reported in seawater, sediment, fish and clams in different sites from the north to the south. *Vibrio alginolyticus* was the dominant bacteria detected in the Tunisian marine environment due not only to its capacity to tolerate hostile environmental conditions but also to its ability to use zooplankton chitin as a nutrient (Zaafraane *et al.* 2022). Nevertheless, the low presence of *V. parahaemolyticus* was attributed to the climate conditions of the southern Mediterranean Sea with high salinity, temperature and sunlight intensity. Some studies confirmed that the presence of *V. parahaemolyticus* is correlated essentially with low salinity (30.9–36.2 psu) (Martinez-Urtaza *et al.* 2008; Caburlotto *et al.* 2016).

Moreover, multidrug resistance strains were recorded especially in sites with high wastewater discharge, which confirm the role of fecal contamination in the dissemination of antibiotic-resistant bacteria in aquatic environments. In fact, overexposure to antibiotics increases levels of resistance in the human commensal microbiota. Subsequently, fecal-resistant bacteria present in environmental settings may disseminate their resistance genes to non-resistant indigenous microorganisms. These genes can encode co-resistance to different classes of antibiotics (Tacão *et al.* 2014). Therefore, more restrictive policies must be recommended for the treatment and assessment of municipal wastewater before its discharge into the sea.

In addition, several studies confirmed the circulation of enteric viruses pathogenic for humans in mollusks such as clams, mussels and oysters. These bivalve species were found contaminated with such viruses like enterovirus, NoV, AsV, hepatitis A

(HAV) and Aichivirus. Bioaccumulation of these viruses in shellfish varies according to the shellfish species and their physiological activity depending on the season and seawater temperature. Especially, the clam's *Ruditapes decussatus* were found more contaminated with hepatitis A than other bivalve species because of their burrowing way of life.

The protozoan parasites of sanitary interest of the genera *Perkinsus*, *Marteilia* and *Bonamia* were also detected in Tunisian bivalve mollusks such as *Ostrea stentina* and *Ruditapes decussatus*. Massive mortality of European flat oyster *Ostrea stentina* along the Tunisian coasts has been attributed to the presence of *Marteilia refringens*.

Tunisian coastal areas are also found to harbor protozoan of human and zoonotic parasites such as *Giardia*, *Toxoplasma gondii* and *Cyclospora*. The detection of these protozoa in clam *Ruditapes decussatus* indicates the contamination of seawater and required careful monitoring procedures. In fact, the bioaccumulation capacity of bivalves could be used to assess the biological quality of seawater. Nowadays, despite the presence of these parasites in marine waters and in the food chain, no national protozoa-monitoring program exists in estuaries and coastal waters.

In general, this review contributed to highlighting the situation of microbiological pollution in Tunisian coastal marine ecosystems and locating the hotspots for microbial pollution. To have a clear viewpoint of the health status more investigations should be conducted especially for viral and parasitic contamination. Nevertheless, improvement of wastewater treatment procedures is highly recommended to reduce the pollutant loads to the sea. In addition, Riparian buffer zones are also suggested to lessen the spread of zoonotic protozoa from pastures to coastlines.

DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

CONFLICT OF INTEREST

The authors declare there is no conflict.

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