A review of the human health risks from microbial hazards in recreational beach sand

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DISCLAIMER

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ABBREVIATIONS

CFU	Colony forming units
FIB	Faecal indicator bacteria
GI	Gastrointestinal illness
HAV	Hepatitis A virus
MST	Molecular Source Tracking
NGS	Next Generation Sequencing
NIWA	National Institute of Water and Atmospheric Research
PCR	Polymerase Chain Reaction
STEC	Shiga toxin-producing *E. coli*
USEPA	United States Environmental Protection Agency
WGS	Whole genome sequencing
WHO	World Health Organization

SUMMARY

Beach sand appears hostile to life, yet microorganisms are abundant. The sand protects them from sunlight, provides colonisable surfaces and traps organic matter and moisture below the sand surface. Beach sand microbial communities are formed from natural inhabitants and those that are introduced, some of which might successfully establish replicating populations.

Bacteria, viruses, parasites and fungi that can cause infection or illness in humans may be naturally found in beach sands or introduced with people or water entering the beach environment. Humans can be exposed to microbiological pathogens in beach sand as they engage in activities that promote skin contact with the sand (rest and play), or through inhalation or ingestion of the sand (play, eating and drinking or playing in shallow waters with high sand suspension). Faecal contamination of beach sand presents the highest risk of beach visitors being exposed to pathogenic microorganisms. Faecal-associated pathogens might contaminate sand from point sources (direct defaecation or via sewage or stormwater outflows) or non-point sources (e.g. surface water run-off).

In 2021, the World Health Organization (WHO) published updated guidelines for assessing and monitoring recreational coastal water quality, which recommended that risk factors for pathogens of concern in beach sand be incorporated into a recreational water safety plan. The WHO recommended intestinal enterococci as an indicator of recent faecal contamination and suggested that additional microbiological guideline values could be set based on local characteristics and an assessment of public health risk. Coastal and marine water quality is monitored in Aotearoa New Zealand using faecal indicator bacteria (FIB) but it cannot be assumed that the presence of these FIB in water reflects the microbial risks from nearby beach sand.

This review was initiated as a first step towards identifying which microbial hazards could pose a public health risk to New Zealand recreational beach users. This review focused on sandy beaches most likely to be visited by people for recreational purposes, where such visits often involve extended time spent in contact with the sand. The focus was also on microbial hazards present in the areas above the tide line (supratidal) and the intertidal zone, with a preference to studies carried out on beaches located in temperate climate zones that are similar to New Zealand.

Sandy beaches are highly variable, even within one beach. They are affected by the movement of terrestrial and marine water and sediment, weather, geology, geography, wildlife and human activities. The concentration and survival of FIB or pathogenic microorganisms in beach sand depends on many factors including the characteristics of the organism, other organisms present, nutrient availability, temperature, moisture, salinity, substrate type and wave energy, plus the type and extent of activities in the environment that contribute to microbial loads (e.g. wastewater or stormwater outflows, bird nesting grounds, agricultural operations, groundwater upwelling and urban areas).

The presence of FIB indicates faecal contamination and the potential for pathogenic microorganisms to be present. *E. coli*, faecal coliforms and enterococci are commonly measured FIB. ‘Hot spots’ of high FIB numbers can occur in beaches if there are point sources of faecal contamination (e.g., wildlife faecal deposits, sewage or contaminated stormwater outflows) or concentrated organic matter (particularly seaweed). The intertidal zone might become a hot spot of contamination when wave energy is low, plus storms can cause FIB concentrations to spike in beach sand. There are some issues associated with using FIB as an indicator; they do not provide information on the source of faecal contamination, die-off rates differ from viral and protozoan pathogens, some species or strains can become naturalised in the environment, and they do not indicate the potential

presence of pathogenic microorganisms from non-faecal sources. However, testing for FIB is a practical method to detect recent faecal contamination.

Molecular techniques can be used to complement FIB monitoring. Metagenomic next generation sequencing, which uses the entire genetic material extracted from a sample, has been used to reveal the diversity of microorganisms present in beach sand, including potential pathogens. This method can detect microbes from faecal and non-faecal sources. Molecular microbial source tracking markers target specific host-associated genetic material to identify sources of faecal contamination. This has been used to identify faecal sources in beaches and support risk mitigation.

Faecal contamination of sand, directly or via faecal-contaminated water, is an important source of many pathogens including zoonotic bacteria (e.g. *Campylobacter* spp. and pathogenic *E. coli*), enteric viruses (e.g. human adenovirus) and zoonotic protozoan parasites (e.g. *Cryptosporidium* spp.). Natural aquatic microbial inhabitants or those that are environmentally widespread, which are also potential human pathogens, can enter beach environments (e.g. *Vibrio* spp., *Pseudomonas aeruginosa*, pathogenic fungi). *Staphylococcus aureus* is an example of an opportunistic pathogen that can be carried into the beach environment by people and subsequently spread into the sand. It has been proposed that total culturable fungi, plus *Candida albicans* and dermatophytes, were useful non-faecal health indicators for beach sand safety. All these microbes, plus some others, have been profiled in this review as potential beach sand hazards. However, there is currently limited evidence to link their presence in sand with adverse human health events, plus data on their presence, concentration and survival in beach sand are scarce.

It is difficult to determine whether people who, after visiting a beach, report adverse health effects such as gastrointestinal infection or skin infections, were infected by microbiological pathogens present in the sand or water. Most people who visit a beach have contact with both sand and water. Children are more likely to experience adverse health effects after visiting a beach and are more likely to actively play in sand. However, children also spend time in the water, particularly the near shore/wave zones. They also generally experience higher rates of gastrointestinal illness compared to adults. Gastrointestinal illness has been linked to playing in sand (sand pits) and there is some evidence that higher concentrations of enterococci in beach sand can lead to higher rates of gastrointestinal infection in beachgoers.

There are no outbreak reports within the scope of this review where beach sand contact was confirmed as the cause of infection. Exposure to beach sand adjacent to a contaminated river might have contributed to an outbreak of cryptosporidiosis, and a contaminated area of sand on a marine beach might have contributed to an outbreak of pathogenic *E. coli* infection.

A quantitative microbial risk assessment, incorporating data on the concentrations of *Cryptosporidium* spp., enterovirus and *S. aureus* in beach sand, predicted that exposure to these pathogens through sand contact carries a low risk of infection. However, the risk will increase if faecal contamination increases pathogen concentrations in sand, or host-associated factors make infection more likely.

In conclusion, microbiological pathogens in sand do present a potential health risk, noting there is limited epidemiological evidence and important data gaps that hinder risk assessment. Further information is required on the presence and survival of FIB and pathogenic microorganisms in sand within a New Zealand context. Enterococci are the recommended indicators for the potential presence of faecal-associated pathogens in marine beach sand. *E. coli* might also be considered as an indicator of faecal contamination in freshwater beach sands. These FIB indicate elevated risk from fresh faecal contamination but do not inform on non-faecal pathogens. Complementary tools, including sanitary surveys and molecular test methods, provide a more complete body of evidence to assess risk.

1. INTRODUCTION

Contact with coastal beach sand has been linked to gastrointestinal illness (GI) and skin conditions (Brandão *et al.*, 2020; Heaney *et al.*, 2009). Pathogenic microorganisms can be introduced into the beach environment from land-based activities or might be natural inhabitants, including those temporarily washed in with seawater. The concentration and survival of pathogenic microorganisms in beach sand depends on many factors including the characteristics of the organism, other organisms present, nutrient availability, temperature, moisture, salinity, substrate type and wave energy, plus the type and extent of activities in the environment that contribute to microbial loads (e.g. wastewater or stormwater outflows, bird nesting grounds, agricultural operations, groundwater upwelling and urban areas).

In 2021, the World Health Organization (WHO) published updated guidelines for assessing and monitoring recreational coastal water quality, which recommended that risk factors for pathogens of concern in beach sand be incorporated into a recreational water safety plan (WHO, 2021). The recommended indicator organisms to assess risk were intestinal enterococci, as an indicator of recent faecal contamination (i.e. the potential presence of pathogenic microorganisms). A guideline value for intestinal enterococci was estimated to be 60 CFU/g (wet weight) of sand, based on rates of sand ingestion by children with *pica* tendencies and assuming that the enterococci:pathogen ratios are the same in sand and water. The WHO suggested that additional microbiological guideline values could be set based on local characteristics and an assessment of public health risk, underpinned by epidemiological studies and quantitative microbial risk assessment. Species of bacteria, viruses, parasites or fungi could all be considered (Solo-Gabriele *et al.*, 2016; WHO, 2021). The WHO recognise that more work is required to establish exposure thresholds for other biological groups and note that a pan-European study has suggested 90 CFU/g of sand (not dry weight) for fungi.

Aotearoa New Zealand (NZ) regional and unitary councils monitor coastal and marine water quality at over 400 sites, most located in the Auckland region and along the East coast of the North Island (Dudley and Jones-Todd, 2018). Currently, there are two microbiological measures used to monitor coastal water quality, these being enterococci and faecal coliforms. The abundance of these bacteria indicates recent faecal contamination and the potential presence of pathogenic microorganisms. The enterococci measure indicates the suitability of the water for recreational contact and the faecal coliform measure indicates whether shellfish are safe to gather (Dudley and Jones-Todd, 2018). However, it cannot be assumed that the presence of these faecal indicator bacteria (FIB) in coastal marine waters reflects the microbial risks from nearby beach sand. The microbial quality of beach sand is not monitored in Aotearoa NZ but there are *ad hoc* studies.

This project has been initiated as a first step towards identifying which microbial hazards could pose a public health risk to New Zealand recreational beach users.

1.1 REVIEW SCOPE

There are a variety of coastal environments that could be described as beaches. In this review, recreational beach sand is considered to be the sand on coastal beach environments most likely to be visited by people for recreational purposes, where such visits often involve extended time spent in contact with the sand, e.g. lying on the beach, children playing with sand. This means that sand beaches are of interest, rather than pebble/stone beaches and mud-like sediments (e.g. estuaries, lagoons/harbours with large intertidal ranges).

This review focuses on microbial hazards present in the sands in the supratidal and intertidal zones. This includes bacteria, viruses, parasites and fungi, either naturally present or introduced via inflows or directly from groundwater, storm events, terrestrial

activities/people/animals, etc. There could also be chemicals entering the beach sand environment through human activities (e.g. wastewater outflows, run-off from agricultural land, landfill leachate, local spills) or natural events (e.g. nitrate from guano, toxins from aquatic algae). Chemical hazards were excluded from this review but might be considered in future work.

It has been recognised that practices such as relocating sand to recreational beaches can introduce microbial hazards, particularly when sand is sourced from estuarine or harbour environments receiving water from catchments with high human activity (e.g. agricultural or urban areas). Similarly, microbial populations in estuarine and harbour sediments can wash into the marine environment and be deposited onto nearby sandy beaches. Just like other types of water outflows (stormwater, wastewater, overland flow), understanding transportation of the estuarine microbial population to nearby beaches requires studies at a local level. So, while this review focuses on the potential for infections by microbial hazards that have been found on sandy beaches, microbial hazards in sediments such as estuarine sand/sediment and liquefaction, have also been considered where relevant data are available for New Zealand.

This review also gives preference to results from studies carried out on beaches located in temperate climate zones that are similar to New Zealand. Thus, studies carried out in the "temperate-without a dry season" (Cf) zone according to the Köppen-Geiger climate classifications were considered to be within scope (Beck *et al.*, 2018).[1] However, this has not been strictly applied; some data from the closely related temperate-dry summer (Cs) and temperate-dry winter (Cw) zones, plus wider subtropical and tropical zones, have been included where they add value (e.g. where data are absent or when studies have important findings). Appendix A includes additional information on the scope of this review.

1.2 REVIEW METHOD

Literature was identified from key documents, scientific citation databases and searches of specific websites. The primary literature search was carried out between August and December 2022 and reflects information available during this period. As described in Section 2.1, the scope of the literature search included microbial hazards in beach sand, and in New Zealand estuarine, harbour and liquefaction sediments.

Two regulatory authorities were selected to provide case studies:

- Greater Wellington Regional Council, who undertake sand testing.
- Auckland Council, where a study of sand contamination has been completed in relation to sewage and combined sewer stormwater overflows, and stormwater as sources of sand contamination.

Appendix A includes additional information on the method.

[1] http://www.gloh2o.org/koppen/ (accessed 23 March 2023)

2. CONTAMINATION OF BEACH SAND

Summary

Sandy beaches are highly variable, even within one beach. They are affected by the movement of terrestrial and marine water and sediment, weather, geology, geography, wildlife and human activities. Microorganisms are abundant in beach sand, which protects them from sunlight, provides colonisable surfaces and traps organic matter and moisture. Beach sand microbial communities are formed from natural inhabitants and those that are introduced, some of which might successfully establish replicating populations.

Microbiological pathogens that can cause infection or illness in humans may be naturally found in beach sands, or introduced with people or water entering the beach environment. Faecal contamination of beach sand presents the highest risk of beach visitors being exposed to pathogenic microorganisms. Many of the relevant human pathogens inhabit the intestinal tracts of animals and are excreted with their faeces. Humans with symptomatic or asymptomatic infections also excrete microbial pathogens with their faeces. These might contaminate sand from point sources (direct defaecation or via sewage or stormwater outflows) or non-point sources (e.g. surface water run-off).

Humans can be exposed to microbiological pathogens in beach sand as they engage in activities that promote skin contact with the sand (rest and play), or through inhalation or ingestion of the sand (play, eating and drinking or playing in shallow waters with high sand suspension). These exposures occur in the dryer supratidal zone and wetter intertidal zone, which is the focus of this review.

2.1 BEACH ENVIRONMENTS

Beach environments are variable and dynamic. They might be in open, coastal environments affected strongly by waves and currents, in protected inlets, coves or harbour mouths, or alongside rivers and lakes. Thirteen types of coastal beaches can be described in terms of their nature: the patterns of waves, tides and currents, and the extent of surf and dry zone features such as bars, troughs and flat areas (NIWA, 2022b). These natural features are influenced by human activities, both at the beach and on adjacent land. The beach environment can be affected by regular or seasonal activities, such as beach grooming or people visiting for recreation, but also by periodic large activities such as the building or demolition of man-made structures, beach sand relocation or dune planting.

A range of generic terms are used to describe the different areas of a beach, such as the foreshore, nearshore and backshore, although the definitions for these zones can differ (NIWA, 2022a; Whitman *et al.*, 2014). For the purposes of this current review, we will consider three main zones that relate to how people are exposed to microbial pathogens while at a beach (Figure 1):

- Intertidal zone: The area between the average low and high tide, also called the swash zone. This is the area that is influenced by waves and tides, and where wet sand is exposed. Wave action can stir sand up into the water. People could be exposed to microbial pathogens in the water and/or sand while in this zone, depending on their activity.
- Subtidal zone: The area below the average low tide mark that is almost always covered in water. The water in this area could still be shallow, depending on the beach morphology, but swimming is a common activity in this area so exposure to microbial pathogens is more likely to be through water contact.

- Supratidal zone: The area above the average high tide mark that is often dry but can be periodically wetted by waves during high tides, or through larger events such as storms and surges. Exposure to microbial pathogens is mainly through contact with the sand.

This current review focuses on exposure to sand microorganisms in the supratidal and intertidal zones. When considering beaches adjacent to non-tidal rivers and lakes, the focus is on exposure to microorganisms in sand in the dry areas of the beach (equivalent to the supratidal zone).

Sandy beaches are permeable and allow water to move through. Water moving underground into beach environments creates a wet sand environment under the surface of the beach, and a groundwater/surface water interface (or groundwater/seawater at coastal beaches, Figure 1). This water movement can introduce and disperse microbial pathogens.

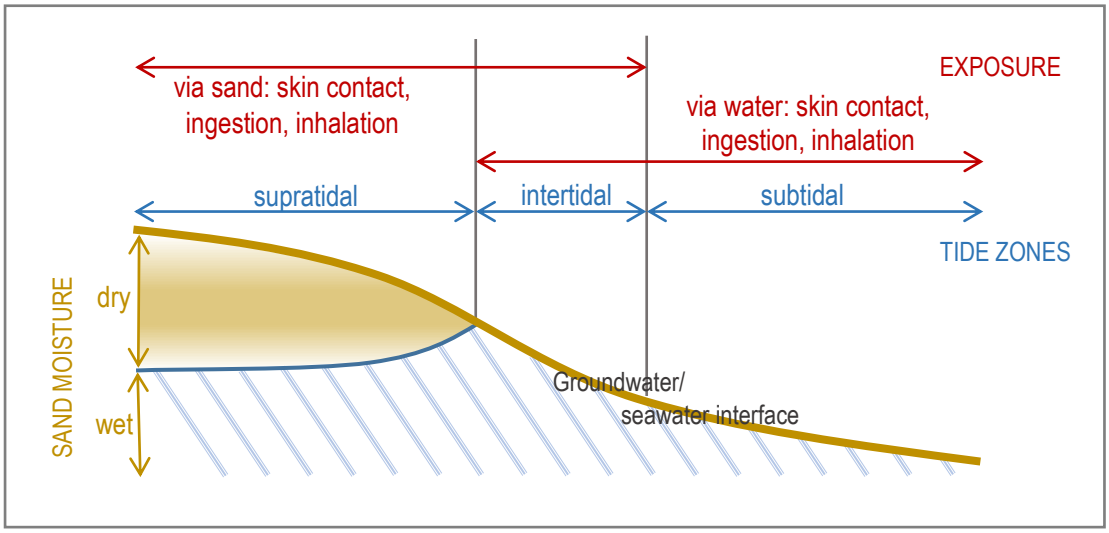

Figure 1: Exposure to microbial pathogens (red) in the three main zones of a coastal sandy beach (blue)

2.2 MICROORGANISMS IN THE BEACH ENVIRONMENT

Beach sand presents a hostile environment, yet microorganisms are abundant. Sand can protect microorganisms against sunlight, trap organic matter and provide colonisable surfaces (Lee *et al.*, 2006). The organisms inhabiting the supratidal and intertidal sands have historically been called the psammon, with the term micropsammon being used for the sand dwelling microorganisms (Whitman *et al.*, 2014). Microbial populations in the sand are natural inhabitants (autochthonous micropsammon) mixed with temporary residents introduced from elsewhere (allochthonous micropsammon) (Whitman *et al.*, 2014). The microorganisms making up these communities vary widely, even across one location (Staley and Sadowsky, 2016). Introduced microorganisms might die quickly, might survive for days or months or, in the case of bacteria, might establish replicating populations and become naturalised. Survival is determined by a range of biotic and abiotic factors, e.g. the type and adaptations of the microorganism itself, the presence of other organisms, nutrients, moisture, temperature, substrate and water movements.

Microbial transport in the beach environment is affected by the direction and energy of water and wind movements (Whitman *et al.*, 2014). They can be planktonic in water (including pore water between substrate grains) or attached to substrates where they can become part of protective biofilms. Porous or fractured sand grains provide a suitable substrate for microbial attachment, as do microplastics and nanoplastics (De Bhowmick *et al.*, 2021; Hernandez *et al.*, 2014). These tiny plastic pieces are environmentally widespread and easily found in New Zealand beach sands (De Bhowmick *et al.*, 2021; de Lena *et al.*, 2021). These plastic particles carry communities of bacteria, including potentially pathogenic species, and work is

underway to measure microbial communities on these substrates under New Zealand conditions.[2]

The region below the surface of the sand is saturated with groundwater, both land-derived freshwater and marine saltwater (Archana *et al.*, 2021). Microorganisms within this space break down organic matter and undertake a wide range of biogeochemical reactions (e.g. denitrification, metal oxidation-reduction).

Because organic matter provides a nutrient source for bacteria, the presence of seaweed in the sand can improve microbial survival and multiplication. Macroalgae blooms occur in New Zealand waters and these can be cast onto beaches through weather, tidal and seasonal events (Nelson *et al.*, 2015). Large accumulations of macroalgal biomass can be a nuisance to beach users and can persist for months as they decompose. However, their decay contributes nutrients to the sediments. Seaweed can also protect microorganisms from sunlight (UV) and desiccation.

2.3 SOURCES OF PATHOGENIC MICROBIAL CONTAMINATION

Microbiological pathogens that can cause infection or illness in humans may be naturally found in beach environments. Examples include windblown fungal spores, pathogens present in faeces from wildlife and waterborne *Vibrio* bacteria. Others are introduced with people entering the beach environment, e.g. carried on their skin or introduced with shoes and vehicles, or present in the faeces of accompanying companion animals). Thirdly, water entering the beach environment through natural pathways or infrastructure might carry pathogenic microorganisms into the sand. There are point and non-point (diffuse) sources of contamination, with the latter being more difficult to identify and control (Brandão *et al.*, 2021).

In terms of human health, faecal contamination of beach sand presents the highest risk of beach visitors being exposed to pathogenic microorganisms. Note, however, that there is currently limited epidemiological evidence connecting human infection or illness with sand exposure (Section 4). Zoonotic pathogens that cause gastroenteritis in humans also inhabit the intestinal tracts of animals and are excreted with their faeces. Humans with symptomatic or asymptomatic infections also excrete microbial pathogens with their faeces.

Point sources of faecal contamination include direct defaecation and areas where faecal matter is concentrated, such as bird nesting areas, horse riding routes and toilets. Dog faeces is an important contributor to beach faecal contamination, containing 100 times more enterococci per gram than wild bird or human faeces, which have similar loads (Wright *et al.*, 2009). Sewage and stormwater outflows from infrastructure are also point sources, although the impact of these on the microbial safety of the sand depends on the quality and dispersal of the discharged water. For example, upgrading the stormwater infrastructure at a beach in Miami, USA, significantly improved sand and water quality, as measured by enterococci concentrations (Hernandez *et al.*, 2014).

Non-point source discharges include other waterways, groundwater or stormwater. These can carry contaminants from land runoff, inland point source discharges and discharges to groundwater such as on-site wastewater systems or effluent irrigation. Urban centres close to recreational beaches can potentially increase non-point source contamination from human activities, e.g. failing sewer infrastructure, run-off from hard surfaces. It can be assumed that animals on farm or tourist operations adjacent to recreational beaches may also contribute non-point source contamination if faecal matter enters the beach area with stormwater, surface water or groundwater flows.

[2] https://www.esr.cri.nz/our-research/research-projects/aotearoa-impacts-and-mitigation-of-microplastics-aim/

3. PATHOGEN INDICATORS

Summary

The presence of faecal indicator bacteria (FIB) indicates faecal contamination and the potential for pathogenic microorganisms to be present. *E. coli*, faecal coliforms and enterococci are commonly measured FIB. There are some issues associated with FIB, e.g. they do not provide information on the source of faecal contamination, die-off rates differ from viral and protozoan pathogens, some species or strains can become naturalised in the environment, and they do not indicate the potential presence of pathogenic microorganisms from non-faecal sources. However, testing for FIB is a practical method to detect recent faecal contamination.

Survival of FIB in sand depends on a range of factors. Abiotic factors include the type of sand substrate, moisture, sunlight irradiation, temperature and nutrient availability, plus beach-associated variables such as tidal range. Biotic factors include the presence of predators and competitors, and the abundance of seaweed deposits.

If there are point sources of faecal contamination, such as wildlife faecal deposits or contaminated stormwater outflows, there will be 'hot spots' of high FIB numbers on a beach. Concentrations of organic matter, particularly seaweed, can also cause hot spots. The variability of beaches means the distribution of FIB will be different between sites and beach zones. The intertidal zone might become a hot spot of contamination on low wave energy beaches, or during periods of low wave energy on surf beaches. Storms can cause FIB concentrations to spike in beach sand.

Molecular techniques can be used to complement FIB monitoring. Metagenomic next generation sequencing, which uses the entire genetic material extracted from a sample, has been used to reveal the diversity of microorganisms present in beach sand, including potential pathogens. This method can detect microbes from faecal and non-faecal sources. Molecular microbial source tracking markers target specific host-associated genetic material to identify sources of faecal contamination. This has been used to identify faecal sources in beaches and support risk mitigation.

3.1 FAECAL INDICATOR BACTERIA (FIB)

FIB are used to indicate a risk to human health from faecal contamination since routine monitoring for the presence of microbial pathogens is impractical unless there is a specific reason. These pathogens tend to be present in a population intermittently and at low concentrations in the environment. Additionally, relative to FIB test methods, pathogen analyses can be complex, have low recovery rates and are expensive. Standard methods might not be available or consistently used. *Escherichia coli* and enterococci are commonly used FIB because they are consistently present in high concentrations in the faeces of warm-blooded animals, although in differing quantities (Wright *et al.*, 2009). There are robust, standard methods available for testing different types of environmental samples, which produce consistent and comparable results.

The presence of FIB in the environment generally indicates recent faecal contamination, and therefore the potential presence of pathogenic microorganisms also excreted with faeces (Devane *et al.*, 2020). This makes these bacteria useful for indicating whether people might be exposed to enteric pathogens from faecal matter in the environment. The use of FIB as an indicator for recreational water quality has led to these being a common microbial target in sand studies, rather than looking for specific pathogenic microorganisms. The WHO have proposed that enterococci be used as an indicator for faecal contamination of sand (WHO,

2021) as it has better survival characteristics compared to *E. coli* which can be used as a freshwater FIB.

Studies of FIB in sand provide clues as to where enteric pathogens might also be found on sandy beaches. This makes it important to include FIB data in this current review. However, there are important issues related to the use of FIB as indicators of faecal contamination:

1. The presence of FIB does not confirm the presence of enteric pathogens, only the potential for these to be present.
2. FIB are indicators of faecal contamination, but not indicators for pathogenic microorganisms that are not excreted in faeces.
3. FIB survival rates can differ from that of pathogenic microorganisms, so the FIB may be poor indictors of the risk from pathogens with longer survival times.
4. The concentration of FIB in the water is not a reliable indicator of the concentration in sand on the adjacent beach.
5. Enterococci appear to be associated with human faecal contamination rather than faecal contamination from mixed sources, with the evidence for this coming from studies of GI from exposure to freshwater.
6. In some conditions FIB can grow and become naturalised in the environment rather than being present in the environment because of recent faecal contamination, potentially triggering 'false positive' results for recent faecal contamination, but still indicative of past faecal contamination.

This current review will not extensively review these points. Some general commentary follows.

Regarding point 1, FIB are common inhabitants of the gastrointestinal tracts of mammals and birds, but their detection does not indicate the source of faecal matter nor confirms that pathogenic microorganisms are present (Korajkic *et al.*, 2018). If, for example, faecal contamination arises from a human source, then there is potential for human pathogens to be present if illness is circulating in the community (Harwood *et al.*, 2014). Alternatively, faecal contamination from cattle, or from wild birds, might introduce different zoonotic pathogens. Through a systematic review, 16 studies were identified that considered the statistical significance of correlations between concentrations of FIB and concentrations or the presence/absence of pathogenic microorganisms in marine/brackish water (Korajkic *et al.*, 2018). No significant relationship was identified in 10 studies while 6 reported a positive relationship between at least one indicator (most often enterococci) and one pathogen (most often adenoviruses, *Salmonella* spp., protozoan parasites or *Campylobacter* spp.). However, such studies tend to be context dependant, e.g. correlations are stronger when FIB concentrations are high and faecal contamination is known to have occurred. As the presence of FIB does not indicate the source of faecal contamination, further investigation is required for source attribution (Walker *et al.*, 2015).

The material assembled in this current review shows that non-enteric pathogens might also be important for sand safety (point 2). Examples include skin and mucous-borne pathogens such as *Staphylococcus aureus*, natural inhabitants of the marine environment such as *Vibrio* spp., and environmentally widespread fungi that are opportunistic human pathogens.

Some pathogenic microorganisms can survive well outside the host (point 3). Parasites that are excreted with faeces but have environmentally resistant life stages, such as *Toxoplasma gondii, Giardia* and *Cryptosporidium* spp., have longer survival times than FIB. A review of *Cryptosporidium* spp. oocyst survival in the environment showed a survival time of 24 weeks in soil at 15°C if protected from desiccation (King and Monis, 2007). Oocyst inactivation rates are faster at higher temperatures, with survival for 12 and 8 weeks at 20 or 25°C,

respectively. In water, decay rates for human viruses are slower than for bacteria (Boehm *et al.*, 2018; Boehm and Soller, 2020). This means that low concentrations of FIB do not necessarily imply a low risk to human health.

The concentration of FIB in sand can be several orders of magnitude higher than the water (point 4) (Abdelzaher *et al.*, 2010; Halliday and Gast, 2011; Sabino *et al.*, 2014). Microcosm studies indicate that FIB could survive longer in sand compared with seawater (Zhang *et al.*, 2015). It has been shown that FIB concentrations in sand and water do not consistently correlate across all studies (De Giglio *et al.*, 2022), although there are studies demonstrating correlations (Phillips *et al.*, 2011). Some studies have found correlations between the concentration of FIB in sands in the intertidal zone and the water, indicating that water quality and sand quality can be related, at least in this zone (Whitman *et al.*, 2014). Microorganisms in the swash zone can move back and forth, although the dominant direction is seaward. Comparisons between FIB concentrations in sand and water should be done with caution, considering these are different matrices, the ratio of FIB:pathogen may differ and they require different approaches to testing.

Considering point 5, a review considering recreational contact with freshwater found that the presence of enterococci in the water, and GI among swimmers, were linked to human sewage contamination (King *et al.*, 2014). Application of the WHO guideline to sand may therefore be more relevant where there is contamination by human sewage.

The concentration of FIB in sand depends on the beach environment itself, the nature and extent of contamination sources and beach visitor activities. Concentrations of *E. coli* or enterococci might exceed 10^3 CFU/100 g or fall below the limit of detection, even along the same beach on the same day (Halliday and Gast, 2011). One study has investigated this variability at a microspatial level, finding that samples of sand taken every 10 cm along a 2 m transect could contain non-detectable levels of enterococci or concentrations as high as $5x10^4$ CFU/100 g (Bonilla *et al.*, 2007). As the following sections show, a range of biotic and abiotic factors combine to influence FIB survival over time, and there can be 'hot spots' of contamination on beaches.

Regarding point 6, the presence of naturalised FIB in the environment has recently been reviewed from the perspective of recreational water quality monitoring (Devane *et al.*, 2020). The authors established that *Escherichia* and *Enterococcus* species have been identified in environmental matrices including soil, sediment and aquatic vegetation. Some species of *Enterococcus* are considered to be truly environmental, while others have adapted to survive outside the host. Some types of *E. coli* have been shown able to survive, and even replicate, in soils and sediments, although another review notes that it is difficult to demonstrate *E. coli* replication in beach environments, where bacterial reintroduction is also a plausible cause of increasing concentrations (Whitman *et al.*, 2014). Evidence from a study of a freshwater lake beach suggested that *E. coli* in the sand had formed a self-sustaining population, at least during summer months, and was perhaps periodically refreshed from gulls or beach visitors (Whitman *et al.*, 2009). A study of 55 beaches along the coast of California concluded that anthropogenic activities are not necessarily important sources of *E. coli*, and that at some beaches these bacteria could be a member of the natural microbial community (Yamahara *et al.*, 2007). Field studies have demonstrated that *E. coli* can multiply in freshwater beach sand but their growth under normal beach conditions is probably limited by competing microflora (Wheeler Alm *et al.*, 2006). Devane *et al.* (2020) conclude that the enteric (faecal) FIB populations might be distinguishable from naturalised populations using genomics but not by current standard FIB test methods. Methods are needed to distinguish between FIB from fresh faeces, aged faeces (e.g. faecal contamination in the past) and naturalised populations.

3.1.1 Environmental factors affecting FIB survival

Moisture, sunlight irradiation, temperature and nutrient availability all interact to influence FIB survival in sand (Whitman *et al.*, 2014).

Moist conditions generally favour microbial survival and bacterial growth. Under controlled conditions using homogenised sand in columns, seawater irrigation appeared to support enterococci multiplication in sand, or at the very least the resuscitation of viable but non-culturable (VBNC)[3] enterococci (Yamahara *et al.*, 2009). The sand and water samples were taken from the same beach, no enterococci were added to the sand, and the irrigation and drainage patterns were designed to align with the natural highest spring tides observed at the source beach. The enterococci were still able to persist in control (non-irrigated) sand columns. In another study, dry sand samples were taken along the Californian coast and it was found that the concentrations of FIB (*E. coli* and enterococci) tended to be higher in sands with relatively higher moisture levels (Yamahara *et al.*, 2012). A longitudinal field study of a freshwater lake beach found the concentration of *E. coli* in sand samples increased after rainfall, attributed to a combination of bacteria entering with stormwater inflows and VBNC bacteria being revived (Beversdorf *et al.*, 2007).

However, not all studies of moisture and FIB are in agreement. Laboratory and field studies have also shown that the concentration of enterococci (but not *E. coli*) in sand is either not significantly affected by moisture levels, or correlates negatively (Mika *et al.*, 2009; Piggot *et al.*, 2012). This is because moisture alone is not the driver for survival, it is just one of the factors.

The sand substrate influences moisture retention. Sands containing higher levels of quartz tend to retain moisture poorly compared to sands containing calcium carbonate (Hernandez *et al.*, 2014). During the beach renovation project in Miami (USA), sand was imported from a quarry (Hernandez *et al.*, 2014). This changed the composition of the beach sand from a mixture of quartz and calcium carbonate to almost entirely quartz, and the concentration of enterococci substantially decreased. While some of this reduction was due to other improvements, particularly to the stormwater infrastructure, it was proposed that the new sand composition did not retain moisture or support biofilms, so enterococci were not retained at the pre-renovation level. Calcium carbonate grains tend to support microbial biofilms better than smoother, quartz grains, and it was observed that the amount of biofilm (as measured by the amount of extracellular polymeric substance extracted from sand samples) reduced after the renovation was completed. Another study found that the dryer sands of the supratidal zone provided a more suitable environment for enterococci to be incorporated into biofilms compared with the intertidal and subtidal zones (Piggot *et al.*, 2012).

Laboratory studies suggest that high temperatures will challenge FIB survival in sand (Beversdorf *et al.*, 2007; Craig *et al.*, 2004; Mika *et al.*, 2009). However, under natural conditions sunlight does not just introduce heat into the sand, but also UV radiation (which damages microorganisms, at least in the top few centimetres of the sand) and a drying effect. Temperature might not be an important driver of survival under natural conditions. As researchers completing a beach study in Miami note, the sand reached 40°C on average but conditions remained suitable for enterococci survival (Abdool-Ghany *et al.*, 2022).

A three-month study of a USA beach (Massachusetts) has demonstrated that there are multiple abiotic environmental drivers for enterococci survival (Halliday *et al.*, 2015). In dry sand, higher enterococci concentrations were associated with:

- Wider tidal range (increases ‘dry’ sand moisture)

[3] Metabolically active cells with potential to become culturable when they encounter more favourable conditions.

- Higher sand moisture (this ranged from <1% to 4%)
- Higher relative humidity
- Lower solar irradiance (i.e. less sunshine)

Tidal range and morning solar irradiance were the most informative variables for predicting enterococci concentration in dry sand. There were no environmental conditions that could predict when wet sand had elevated enterococci concentrations, although wind appeared important through causing higher wave runup and recirculation of enterococci in the swash zone. This study also showed that enterococci concentrations were, overall, higher in dry sand compared to wet sand, and higher in sand compared to water. Concentrations in sand increased during periods of wet weather.

FIB can also be eaten by microfauna such as protozoa and nematodes, and face competition with other sand microflora for nutrients (Devane *et al.*, 2020; Whitman *et al.*, 2014). In sediment microcosm experiments, FIB survived better in sterilised sand compared to non-sterilised sand (Hartz *et al.*, 2008; Korajkic *et al.*, 2013). Competition with autochthonous sand bacteria might be more important than predation (Feng *et al.*, 2010).

Nutritious organic matter can be washed into the sand from the land or water, including large deposits of algae (seaweed, wrack). These encourage bacterial survival and growth by providing nutrients and protection from sunlight. A study in New Zealand found high concentrations of enterococci in beach seaweed and a significant association between enterococci levels measured in the sand and in the seaweed, plus provided laboratory evidence to show enterococci multiplication in seaweed (Anderson *et al.*, 1997). A study in California detected enterococci and *E. coli* in samples of mixed macroalgae wrack taken from the dry areas of beaches, at higher concentrations than wrack taken from wet and surf zone areas of the beaches (Imamura *et al.*, 2011). Almost consistently higher concentrations of these FIB were also detected in sand samples taken from underneath the dry sand wrack compared to sand samples taken away from the wrack. Further studies using microcosms confirmed that the presence of wrack supported FIB survival and growth in sand (Imamura *et al.*, 2011).

Beach management practices can also affect FIB. Grooming of beach sands was found to reduce *E. coli* concentrations in the sand and improve the quality of the water (Kinzelman *et al.*, 2004; Kinzelman *et al.*, 2003).

3.1.2 'Hot spots' of contamination

As Section 2.3 describes, there will be hot spots of contamination on a beach where there are point sources of faecal contamination. For example, one study found that enterococci from a single faecal deposit from a seagull could radially migrate in dry sand, contaminating an area of $3m^2$ (Bonilla *et al.*, 2007). The data on FIB were reviewed to see whether some zones of the beach (Figure 1) were more likely to retain microorganisms.

A review published in 2014 describes a range of laboratory and field studies that examined the fate of *E. coli* and enterococci in beach environments (Whitman *et al.*, 2014). The review shows that the results from studies comparing the density of FIB in different beach zones, relative to the sea, are inconsistent. Some studies found higher FIB densities in wet foreshore sand compared to dry backshore sand, others found higher concentrations in lower moisture, supratidal sands. More recent studies continue to show differences between beach zones (Cloutier and McLellan, 2017; Cui *et al.*, 2013). This means that it is difficult to predict which beach zone will have higher FIB concentrations, i.e. every beach will be different. This is not unexpected considering the variability of beach environments.

Despite the above, several studies indicate that the intertidal zone might become a hot spot of contamination on low wave energy beaches, or during periods of low wave energy on surf beaches. Sand can serve as a vehicle for transferring pathogenic microorganisms to the

water, and can itself become contaminated by the water (Solo-Gabriele *et al.*, 2016). This mixing occurs through water movement (wave action, currents, interstitial pore water transport). Field experiments using synthetic microspheres as a proxy for bacteria indicated that high energy wave action in the swash zone can rapidly move non-attached (planktonic) bacteria deeper into the sand, and horizontally seaward (Gast *et al.*, 2015). In contrast, beaches with lower wave energy and longer slopes can allow bacteria to settle, aggregate and perhaps multiply (Feng *et al.*, 2016). A temporal study of a freshwater beach in Canada, where periods of high wave intensity can occur, recorded lower concentrations of *E. coli* in foreshore sand samples during periods of higher wave heights (Vogel *et al.*, 2016). For the fine sand beaches, erosion of the sand into the water was the primary *E. coli* transport process. Interstitial pore water transport appeared to be more important on coarser sand beaches.

Further evidence for the above comes from surveys that have found higher concentrations of FIB in the wet sand of sheltered beaches compared with open beaches (Lee *et al.*, 2006; Yamahara *et al.*, 2007). In one study, the concentrations of enterococci were 2-3 times higher in sand from two enclosed beaches with low wave activity compared to 13 open beaches (noting that the same trend was not evident for *E. coli* and the authors do not describe other factors that might influence enterococci levels) (Lee *et al.*, 2006). In a second study, at 55 beaches along the Californian coast, composite sand samples were taken from the dry area above the tide line, the wet swash zone, and a targeted area where FIB levels were likely to be elevated (e.g. adjacent to a stormwater outflow) (Yamahara *et al.*, 2007). Enterococci levels were higher in the wet and targeted sand samples but more importantly, the absence of wave action was significantly associated with the presence of enterococci and *E. coli* in beach sands. The concentration of these FIB was also influenced by the presence of a potential FIB source (e.g. stormwater drain or river) and the percent urban and agricultural land use within 10 km of the sampling site. The small/absent tidal range and lower wave energy on the shore of many freshwater lakes means sand in these zones can become a microbial sink, developing a microbiological population diversity that differs from that in the water or submerged sediment (Nevers *et al.*, 2020). A study of two freshwater lake beaches in Canada found higher *E. coli* concentrations in dry sand collected from the foreshore, near the water's edge, than in sand samples from upshore areas (further from the water) and submerged areas (Staley *et al.*, 2015).

Concentrations of organic matter can also cause hot spots. As explained in Section 2.2, the presence of seaweed can support microbial survival and growth. Another study has shown that, in the absence of beach visitors, enterococci concentrations in the sand were highest in areas of the beach with stranded seaweed (Abdool-Ghany *et al.*, 2022). At times, concentrations exceeded the provisional guideline of 60 CFU/g enterococci (WHO, 2021). When beach visitors returned, enterococci concentrations in other parts of the beach increased, particularly in the supratidal zone, and this was partly attributed to seaweed being spread by foot traffic. It was thought that enterococci were being washed out of the sands in the intertidal and subtidal zones. An earlier New Zealand study showed that seaweed can be a source of enterococci in beach environments (Anderson *et al.*, 1997).

Extreme weather events can cause FIB concentrations to spike in the upper areas of beaches. The concentrations of enterococci and *E. coli* in sand from the landward edge of a Japanese beach was highest immediately after a typhoon, and these bacteria were detected in all layers of the sand, down to 100 cm (Suzuki *et al.*, 2018). These FIB were undetectable one month later. A temporal study of a beach in South Australia detected much higher FIB concentrations in sand samples taken during a storm event compared with all other samples taken during a three-month period (Whiley *et al.*, 2018). The concentrations had returned to average levels within a week following the storm. Similarly, FIB concentrations spiked in beaches of the Archipelago of Madeira (Portugal) after an extreme rainfall and flash flooding event (Abreu *et al.*, 2016), and in the sand of open beaches in Southern California (USA) during a storm (Lee *et al.*, 2006).

3.2 OTHER POTENTIAL INDICATORS OF FAECAL CONTAMINATION

E. coli and enterococci have been used as FIB to assess the quality of recreational waters for over a century (Korajkic *et al.*, 2018). Alternative or complementary tests are now available. New analytical techniques have been developed by molecular biologists that target genetic material from microorganisms. When these methods are designed to analyse all the genetic material in a sample, to determine which organisms are present, this is called metagenomics. Metagenomic methods address some of the FIB weaknesses noted in section 3.1, since the methods can detect multiple microorganisms in one test and indicate the likely sources of contamination. However, these molecular techniques only detect gene fragments and do not indicate whether this genetic material came from viable microorganisms, nor do they quantify the microorganisms present. In contrast, microscopy with DAPI staining or culture techniques enable live microorganisms to be measured but the methods are microorganism-specific and some microorganisms (e.g. many viruses), are not able to be cultured to prove they are viable.

Metagenomic methods have been used to identify microorganisms. Metagenomic Next Generation Sequencing (NGS) is a high-throughput method that amplifies and sequences short pieces of extracted DNA or RNA in a sample. The sequences are compared with a library of genetic data to identify the microorganisms present. The relative abundance of different microorganisms can be calculated. In a study using NGS, a diverse range of bacteria was detected in sand samples from recreational beaches in South Africa (Sibanda and Ramganesh, 2021). The dominant genera were: *Bacillus*, *Bifidobacterium* and *Lactobacillus*. Upon further analysis the researchers concluded that non-pathogenic bacteria were most abundant, but noted that the presence of probiotic bacteria could indicate the presence of faeces, since these bacteria are naturally found in the intestinal tract of humans and animals. An interesting finding was the bacteria *Achromobacter xylosoxidans*, which is an emerging, multidrug-resistant opportunistic pathogen causing infections in immunocompromised patients. This suggests sand could be a reservoir of antibiotic resistance genes. This study shows an advantage of NGS in that it can detect microbes from faecal and non-faecal sources.

Microbial Source Tracking (MST) uses qPCR (quantitative Polymerase Chain Reaction) to target specific host-associated genetic material in the gut of animals and humans. The concentration of these markers can be calculated. MST is used to identify the sources of faecal contamination by targeting and quantifying genetic markers of species-specific microorganisms or host-bacteria interactions. Some markers can additionally indicate aged faecal contamination. For example, the human marker crAssphage has a slower decay rate than FIB and bacterial MST markers, and its continued presence when there are very low concentrations of other human MST markers is considered indicative of aged or treated sewage (Boehm *et al.*, 2018; Leonard *et al.*, 2021). A 2022 publication describes how MST was used to determine the contamination sources in the supratidal sand of a coastal beach located in Portugal (Valério *et al.*, 2022). The work identified that dog faeces were one important source of contamination, which helped inform mitigation measures. A series of studies of freshwater sands from Lake Michigan identified a correlation between *E. coli* concentrations and the MST marker for gull faeces, providing evidence that wild birds were an important source of faecal contamination in that environment (Nevers *et al.*, 2020).

In an Australian study, MST markers were investigated for their use as indicators for human wastewater contamination of beach environments (Hughes *et al.*, 2017). While most of this work focused on water, the human faecal marker HF183 was detected in some sand samples from Queensland beaches. The sand samples were not tested for culturable FIB and PCR inhibition was an issue. Another study, carried out in the USA, detected markers for gull faeces in beach sand samples but this did not correlate well with gull markers in paired water samples, nor with the presence of FIB (Cloutier and McLellan, 2017).

A study in California attempted to identify genetic markers that could be epidemiologically linked to GI in swimmers (Griffith *et al.*, 2016). The researchers evaluated 67 different methods that together targeted 41 indicators. While this study only focused on water samples and GI among swimmers, the comprehensive methodology revealed some important findings that could apply to sand:

- Most of the methods did not perform well, with few indicators being associated significantly with gastrointestinal disease.
- A USEPA standard method (EPA Method 1600) targeting enterococci by culture performed most consistently.
- One promising indicator was F+ coliphage by culture, under high-risk conditions at two beaches. At the third beach qPCR F+ coliphage was found to be associated with illness, but not under high-risk conditions. However, F+ coliphage was not measured in sediment or sand. It is noted that F+ coliphage was not associated with illness in other studies (Heaney *et al.*, 2012; Wade *et al.*, 2010). F+ coliphages are viruses that infect *E. coli*, which might survive similarly in the environment to viruses excreted by humans in faeces.
- MST markers of human faeces were only predictive of GI at one site known to be impacted by human sewage from faulty infrastructure.
- Composite indicators might be a viable alternative, whereby a detection score is calculated based on the number of indicators detected in a sample combined with the number of indicators present in that sample at a concentration more than ten times the detection limit.

The WHO recreational water quality guidelines note that MST is a useful tool but there is no consistent methodology (WHO, 2021). WHO recommend multiple lines of evidence before making inferences. A robust well-defined methodology is important to ensure that the results are consistent, which allows comparison between data sets. It was considered that significant knowledge of MST was required to use these tools in a study to trace faecal contamination, especially where there are multiple sources.

4. ADVERSE HEALTH EVENTS

Summary

It is difficult to determine whether people who report adverse health effects, such as gastrointestinal infection or skin infections, after visiting a beach were infected by microbiological pathogens present in the sand or water. Most people who visit a beach have contact with both sand and water. Children are more likely to experience adverse health effects after visiting a beach and are more likely to actively play in sand. However, children also spend time in the water, particularly the near shore/wave zones. They also generally experience higher rates of gastrointestinal infection compared to adults. GI has been linked to playing in sand (sand pits) and there is some evidence that higher concentrations of *Enterococci* in beach sand can lead to higher rates of gastrointestinal infection in beachgoers.

A quantitative microbial risk assessment, incorporating data on the concentrations of *Cryptosporidium* spp., enterovirus and *Staphylococcus aureus* in beach sand, predicted that exposure to these pathogens through sand contact carries a low risk of infection. However, the risk will increase if faecal contamination increases pathogen concentrations in sand, or host-associated factors make infection more likely.

There are no outbreak reports within the scope of this review where beach sand contact was confirmed as the cause of infection. Exposure to beach sand adjacent to a contaminated river might have contributed to an outbreak of cryptosporidiosis, and a contaminated area of sand on a marine beach might have contributed to an outbreak of pathogenic *E. coli* infection.

4.1 SAND EXPOSURE AND EPIDEMIOLOGICAL STUDIES

People are exposed to pathogenic microorganisms in the sand through direct skin contact (dermal contact), or when sand goes into the eyes, ears, lungs or mouth (Solo-Gabriele *et al.*, 2016). Transfer of sand from hand-to-mouth occurs frequently as beach visitors eat, drink, and play in the sand or at the sand–water continuum (Brandão *et al.*, 2022). The foreshore and intertidal zones of beaches are the areas where beach goers tend to congregate, particularly children.

Studies of the behaviour of recreational beach visitors confirm that children tend to have greater active contact with the sand compared to teenagers and adults, digging and being buried in sand (DeFlorio-Barker *et al.*, 2018; Ferguson *et al.*, 2019). One study observed that children aged 2-3 years were more likely to bury themselves in sand compared to other children aged six and younger (Ferguson *et al.*, 2019). However, age was not important when respondents were asked about eating or drinking after playing in sand, nor about washing their hands after playing in sand. Over half of the respondents reported consuming food/drink and approximately 40% reported washing hands (although the method of washing was not described) (DeFlorio-Barker *et al.*, 2018).

A laboratory study has demonstrated *E. coli* can be transferred from beach sand to hands (Whitman *et al.*, 2009). The *E. coli* measured in these trials were present in sand collected at different time points from a freshwater beach, rather than being added in the laboratory. Higher concentrations of *E. coli* in sand resulted in higher concentrations on the hands, although the participants did not brush sand off their hands before hand concentrations were measured by rinsing. Further sand-to-hand transfer studies were conducted using sand spiked with an F+ coliphage (MS2), showing viral transfer was also possible. Importantly, these studies showed that hand rinsing once removed the majority of *E. coli* (86% or more)

and coliphage (96% or more). Thus, rinsing hands under clean water effectively reduces the risk of pathogen ingestion along the sand-hand-mouth pathway.

Wound infections might also occur from exposure to pathogenic microorganisms in sand. Infection can occur through recent wounds and through wounds occurring while at the beach. In a study of 122 children aged 6 years and under, 58% had at least one existing abrasion before they played at the beach and 8% acquired a new abrasion during their time at the beach (Tomenchok *et al.*, 2020). A New Zealand review of 4407 incidents where children (<16 years old) required first aid by surf lifeguards during the period 2007-2009 found that 44% concerned lacerations/abrasions (Moran and Webber, 2014).

Of most value to assessing the risk posed by sand are studies that not just record behaviours specifically associated with beach sand and water contact, but also whether visitors experienced any adverse health effects on the beach day or during subsequent weeks. One problem is that most recreational beach visitors come into contact with the water (wading, swimming) as well as the sand, so if they experience adverse health effects it can be difficult to determine whether this occurred from exposure to water or sand. This problem was evident in a systematic review and meta-analysis that found relationships between beach sand contact and adverse health effects were non-significant or inconclusive (Russo *et al.*, 2020).

However, a subset of each study population are often non-swimmers and data from this group can be compared to that of swimmers if the data set is large enough.

Over a period of nearly 10 years, data collected through the US National Epidemiological and Environmental Assessment of Recreational Water (NEEAR) study were used to investigate links between recreational beach exposure and illness. During 2003, 2004, 2005, 2007 and 2009, researchers prospectively enrolled visitors to a range of freshwater and marine beaches and asked them about their behaviours while at the beach on the day, then followed up 10-12 days later by telephone to ask about any new health problems. From a dataset of 54,250 interviews of visitors to four freshwater beaches and five marine beaches in the USA, it was found that swimmers tended to report new health symptoms more often than non-swimmers (Collier *et al.*, 2015). In this combined cohort, 13.5% of non-swimmers reported at least one new health symptom in the 10-12 day period after the beach visit, compared with 16.6% of swimmers. While interaction with sand by non-swimmers was not reported, the incidence of illness is greater than the 5% calculated as a background incidence of GI by the WHO (WHO, 2003). The adverse health symptoms reported were GI, respiratory illness, ear problems and rash, with GI being the highest amongst non-swimmers (overall 5.5%).

Some of the publications arising from the NEEAR study focused on sand contact as a risk factor. Of the total 54,250 visitors, 27% did not enter the water and 35% dug in the sand while visiting (Collier *et al.*, 2015). From the combined data for 2003-2005 and 2007 (seven beaches, 27,365 interviews), it was found that digging in the sand or being buried in the sand were positively associated with gastrointestinal illness and diarrhoea (Heaney *et al.*, 2009). There was no association between sand contact activities and non-enteric illness (respiratory illness, rash, ear or eye ailments, infected cuts). In this study swimmers and non-swimmers were not analysed separately, and the authors did not report probability values, so the findings should be viewed as indicative only.

The 2007 cohort is perhaps the most informative study, since during this project the researchers collected wet sand samples from the beaches as well as data from visitors (Heaney *et al.*, 2012). This work targeted two marine beaches, collected data from 4,999 visitors and tested 144 wet sand samples for *Enterococcus*, *Bacteroidales*, faecal *Bacteroides*, *Clostridium* spp. and F+ coliphage by PCR. *Enterococcus* and F+ coliphage were also analysed by culture. Unfortunately, the numbers of participants who did not swim, but who dug in sand or were buried in sand, were low (257 and 24, respectively). This meant

that the number of reported health affects was too low among the non-swimmer group to enable meaningful analyses. Thus, the researchers did not analyse the non-swimmer group separately but did note this weakness in their study. There were three relevant findings:

- There was an increased risk of illness for those who reported digging in sand containing higher enterococci and *Bacteroidales* concentrations. People who reported getting sand in their mouth were also more likely to experience GI when the enterococci sand concentrations were higher.
- The association between the concentration of enterococci in sand and GI was more consistent when PCR-based methods were used for the sand testing, compared to traditional culture-based methods. As mentioned before this could be because qPCR measures all genetic material present, whether or not the enterococci is culturable.
- The other microbial indicators, using culture or PCR-based methods, were not significantly related to GI.

As noted previously, the concentration of enterococci and the incidence of GI correlate better when human sewage is the source of faecal contamination at the location of exposure.

Another study has relied on self-reported data obtained from adults through a web-based survey (Leonard *et al.*, 2020a). Participants were asked to retrospectively report any exposure to coastal waters in England and Wales in the previous two weeks[4], and any symptoms of illness. Of 2,631 respondents, 412 were beach-going non-swimmers, 1,693 were beach-going swimmers and 526 (a control group) were non-beachgoers. Those who swam were more likely to report skin or ear ailments than non-swimmers (beach-going and non-beachgoers, combined), but the proportion of swimmers reporting other ailments such as GI or respiratory infection was not significantly different to non-swimmers. Of more relevance to this current review, the researchers found that beach-going non-swimmers were not significantly more likely to experience ailments compared to non-beachgoers. For example, 2% (8/412) of beach-going non-swimmers reported GI during the seven-day recall period compared to 3% (17/526) of non-beachgoers (adjusted odds ratio 0.56, 95% confidence interval 0.24-1.31, p=0.18). This incidence of illness in the non-beachgoing cohort is a less than the 5% calculated by WHO (2003), but the survey excluded children who have higher rates of illness. However, the researchers did not collect data on sand contact.

Using NEEAR methodology, a more recent study investigated norovirus infection among beach visitors who swam (head immersion) and non-swimmers, in Puerto Rico (Wade *et al.*, 2018). The results indicated that contact with water was more important than contact with sand and the most impacted group was children 5-11 years of age. Using a saliva immunoassay that indicates a recent norovirus infection, no norovirus infections were identified among 199 non-swimmers during subsequent weeks. In comparison, 3.4% of the 903 swimmers were positive, almost all of whom were asymptomatic. The study does not report if the ages of the non-swimmers and swimmers are similar.

As part of a study of *Staphylococcus aureus* in Florida beaches, beach visitors were invited to submit information on "occurrences of gastrointestinal, constitutional, dermatological, or upper respiratory illnesses" within four days of visiting the beach (Esiobu *et al.*, 2013).[4] From 882 completed questionnaires, plus 609 completed questionnaires from non-beachgoers (control group), it was found that skin conditions were reported by 18% of beachgoers compared to 11% of non-beachgoers (the statistical significance is not reported). The mean concentrations of *S. aureus* in sand samples grouped by type or location were $>10^3$ CFU/g. While there appears to be a health risk associated with visiting these beaches, the authors advised against making this interpretation due to the low survey response rate. The use of

[4] NEEAR studies use a reporting period of 10-12 days

control groups who are not beachgoers has been criticised by some, who question whether the overall health status of beachgoers and non-beachgoers should be considered equal (Bonilla *et al.*, 2007).

The above studies focus on the health of beach visitors. However, the authors of a 2014 review point out that the health of workers who are in frequent contact with sand requires special consideration (Sabino *et al.*, 2014). In New Zealand, the most obvious occupationally exposed group are lifeguards.

4.2 QUANTITATIVE MICROBIAL RISK ASSESSMENTS (QMRA)

Weiskerger and Brandão (2020) have reviewed QMRA studies published since 2015 that investigate the risks from pathogenic microorganisms through exposure in beach environments or swimming pools. With the exception of two studies (described below), these focus on recreational exposure to pathogenic microorganisms in water while swimming or participating in other recreational activities.

One study considers GI from exposure to adenovirus through contact with sand through ingestion and inhalation (Kundu *et al.*, 2013). However, the endpoint, an individual's illness risk (IIR) from sand contact, was calculated using data relevant to adenovirus exposure through water ingestion and therefore this is not considered a good model.

A second QMRA has carefully considered exposure scenarios for sand, taking into account exposure to pathogenic microorganisms via oral and dermal pathways (Shibata and Solo-Gabriele, 2012). Multiple factors are investigated within each pathway. The hand-to-mouth pathway considers the exposure duration and either a single value ingestion rate (g/h), or ingestion calculated from values for the surface area of the skin mouthed, sand to skin adherence, hand-to-mouth frequency and transfer efficiency from hand to mouth. The dermal pathway considers either an exposure scenario when only some contacted sand remains on the skin, or when all of the sand remains on the skin and is evenly distributed. The QMRA model was used to calculate the concentration of *Cryptosporidium*, enterovirus or *S. aureus* in sand that would be necessary to result in a risk of 1.9×10^{-2} illnesses per visit. This is the USEPA acceptable level of risk from gastrointestinal illness for swimming in marine recreational waters (19 cases per 1000 swimmers). The oral pathway was applied for exposure to *Cryptosporidium* and enterovirus, and the dermal pathway to *S. aureus*. Data on the concentrations of these pathogens in sand were incorporated into the exposure assessment. Considering the different equations applied, and illness risks at the 25th and 50th percentiles, the following concentrations were reported to result in a risk of 1.9×10^{-2} illnesses per visit:

- 10-1000 oocysts/g sand of *Cryptosporidium* via oral exposure.
- 5-500 MPN/g sand of enterovirus via oral exposure.
- 10^6-10^7 CFU/g sand of *S. aureus* via dermal exposure.

The measured sand concentrations of these pathogens were much lower than the above values, e.g. up to 0.12 oocysts/g for *Cryptosporidium*. While this suggests the risk from sand is low, the authors point out that faecal contamination will raise sand *Cryptosporidium* and enterovirus concentrations, potentially to the above levels. For *pica* children who consume non-food items, the sand concentrations of *Cryptosporidium* or enterovirus per gram of sand were predicted to be very low to reach the benchmark risk level (1-3 oocysts/g or MPN/g, respectively). The authors also note that the dose response relationship used for *S. aureus* was for intact skin. This risk of infection is likely to be higher for individuals with cuts or wounds. Overall, the outputs are considered conservative, since the QMRA uses infection rates as a proxy for illness rates. Not all infections result in illness.

A recent review has concluded that data for fungal pathogens are currently insufficient to develop a QMRA for beach sand exposure (Weiskerger and Brandão, 2020). An important data gap is dose response.

4.3 OUTBREAK REPORTS

4.3.1 Outbreaks from exposure to beach sand

Only two outbreak reports were located where exposure to sand was a potential cause of microbiological infection but neither confirmed sand contact as a risk factor.

A 2013 outbreak of cryptosporidiosis in the city of Halle, Germany, involved people (particularly children) who had visited playgrounds, picnic areas and a beach adjacent to the city's main river (Gertler *et al.*, 2015). The outbreak began six weeks after the peak of an extensive river flooding event that damaged sewage systems. Of the 167 cases identified, 24% were classified as potential secondary cases. Oocysts were found in water samples, persisting months after the flood events, but it was not established whether they were surviving in the sediment following flooding or being continuously introduced with sewage overflows. Samples from the beach were not tested.

An outbreak of *E. coli* O157 infection in the UK was linked to the same beach in Devon (Harrison and Kinra, 2004). Foodborne transmission was eliminated and contact with the beach environment was the only plausible risk factor since the cases had all occupied the same part of the beach on the same day. This was unable to be confirmed because *E. coli* O157 were not detected in sand samples nor seawater samples. *E. coli* were detected in some samples from a nearby estuary, although the isolates were not the outbreak strain. This environmental testing occurred more than a week following the likely exposure date. A raw sewage spill had occurred one mile from the beach, during the month prior to the outbreak, but this was not considered to be the cause.

Other outbreak reports were located which were out of the scope of this current review because of the causative agent (e.g. a chemical or vectorborne pathogen) or the transmission pathway. They are described below to provide a complete record of beach-associated outbreaks, for future reference.

Cryptococcosis is a disease caused by the yeast *Cryptococcus gattii*. This yeast can infect immunocompetent humans and animals, usually through inhalation of the fungal spores. An outbreak was reported among humans and animals who were exposed to the yeast in the coastal environment of Vancouver Island, Canada (Kidd *et al.*, 2004). Genomically-linked yeast isolates were found in the coastal forests showing airborne transmission was the likely pathway of infection. This suggests that exposure was not through contact with sand, although beach sand samples were not tested. Transmission to the marine environment had occurred since marine mammals were among the diagnosed cases. *C. gattii* was considered to be a pathogen of tropical and subtropical climates but this outbreak, in a cooler region, has changed that view.

One report details an outbreak of viral skin infections among beach volleyball athletes (Tertipi *et al.*, 2021). However, the human-associated viruses (herpes simplex, molluscum contagiosum and human papilloma) could have been spread through skin contact, wet sand and/or volleyballs, so the role of sand was not clear. In addition, the researchers did not investigate athlete hygiene nor shared environments such as changing rooms.

One often cited beach-associated outbreak, occurring during 2019, involved 29 children and one adult who developed macular erythematous pruritic skin rash two days after sifting sand at the Portuguese island of Azores (Brandão *et al.*, 2020). This was linked to faecal contamination, but the causative hazard was most likely chemical rather than microbiological. Dry sand testing yielded concerning levels (>201 MPN/g) of total coliforms, *E. coli* and enterococci, indicative of faecal contamination. However, elevated levels of

sodium hypochlorite were also present in sand samples and the identified cause was failed sewage infrastructure. The rash was probably caused by sodium hypochlorite, used for surface and toilet cleaning, rather than microbial contamination. None of the cases reported GI symptoms.

A fourth report describes an outbreak of murine typhus among beach sunbathers in Athens, Greece (Labropoulou *et al.*, 2021). The causative bacteria, *Rickettsia typhi*, is endemic in some parts of New Zealand (Lim *et al.*, 2016b; Roberts and Ellis-Pegler, 2001).[5] However, the disease is vectorborne since *R. typhi* is usually transmitted by the rat flea, *Xenopyslla cheopis*. The outbreak was probably caused by rats attracted to litter on the beach, which allowed the fleas to temporarily migrate into the sand.

4.3.2 Outbreaks from exposure to sand

Outbreaks that have occurred from exposure to sand in non-beach conditions provide evidence to show the potential for illness through active sand contact, without the complication of potential exposure to contaminated water.

Salmonellosis has been linked to contact with playground sand in Australia, Spain and the Netherlands (Doorduyn *et al.*, 2006; Lucerón *et al.*, 2017; Staff *et al.*, 2012). Contamination with faecal matter from wildlife was the confirmed cause of the Australian outbreak, and nesting birds were the probable source for the outbreak in Spain. Sources of contamination were not investigated in the Netherlands study, which used a case control study to investigate salmonellosis risk factors.

One outbreak, occurring in 1977, demonstrates the potential for toxoplasmosis to result from exposure to contaminated substrates. Thirty-seven people developed toxoplasmosis after a horse-riding event and, while all substrate samples were negative, positive results from cats and mice in the stable strongly suggested that the soil, sand and/or sawdust had been contaminated with oocyst-containing cat faeces (Dubey, 2021; Teutsch *et al.*, 1979).

[5] https://www.health.govt.nz/our-work/diseases-and-conditions/communicable-disease-control-manual/rickettsial-diseases

5. MICROBIAL HAZARDS

Summary

Faecal contamination of sand, directly or via faecal-contaminated water, is an important source of many pathogens including zoonotic bacteria (e.g. *Campylobacter* spp. and pathogenic *E. coli*), enteric viruses (e.g. human adenovirus) and zoonotic protozoan parasites (e.g. *Cryptosporidium* spp.). Natural aquatic microbial inhabitants or those that are environmentally widespread, which are also potential human pathogens, can enter beach environments (e.g. *Vibrio* spp., *Pseudomonas aeruginosa*, pathogenic fungi). *Staphylococcus aureus* is an example of an opportunistic pathogen that can be carried into the beach environment by people and subsequently spread into the sand. All these microbes, plus some others, have been profiled in this section as potential beach sand hazards. There is currently limited evidence to link their presence in sand with adverse human health events.

For most of the microbiological pathogens, data on the presence, concentration, viability and survival are scarce or absent. It is also difficult to make generalisations from the available data since beach environments, and their microbial communities are highly variable.

It has been proposed that total culturable fungi, plus *Candida albicans* and dermatophytes, were useful non-faecal health indicators for beach sand safety. However, further evidence is needed that links beach sand concentrations of fungi with adverse human health effects.

A range of microbial hazards might be present in beach sand. Of interest are pathogens introduced into sand from faeces (including via water) or beach visitors (e.g. skinborne pathogens), plus those naturally present in New Zealand's coastal environments. Relevant pathogens need to have the potential to cause adverse health effects if they are ingested, inhaled, or come into contact with skin.

This chapter summarises the relevant characteristics of microbial pathogens that are more likely to be important for New Zealand based on the likelihood of them being present in beach sand and the likelihood that they could cause infections in humans through this exposure. Influencing this list was also the assumption that faecal contamination of beach sand presents the greatest hazard to human health. Further information on how pathogens were selected or excluded is given in Appendix B. While the listed pathogens have been selected based on published literature, public health surveillance and geography, there is currently no robust evidence to link their presence in sand with adverse human health events:

- There are few published studies of pathogenic microorganisms in beach sand compared to beach water. Such studies often focus on identifying pathogens at the genus level rather than identifying species or types that are known human pathogens (Whitman *et al.*, 2014). These studies do not link pathogen presence with human infection via sand exposure.
- Outbreak data are not informative (Section 4.3). Outbreaks from recreational water contact are comparatively more common but the hazard exposures and risks are not the same for non-swimmers (Section 4.1).

Information on the abundance and survival of the selective microbiological pathogens in beach sand has been included where available. In general, pathogenic bacteria and yeasts might multiply outside their hosts if conditions are suitable, although data on their behaviour

in beach sand are limited. Viruses and protozoan parasites require a host to multiply but many survive well in the environment. However, as discussed in earlier chapters of this review, the environmental conditions (particularly moisture) that affect the survival of microorganisms and beach environments are highly variable. This means that the information on pathogen presence and survival in sand that is presented in the following sections is very contextual. High level information on survival in soils provides some indication of what could be possible in sand at 20-30°C (WHO, 2006):

- Bacteria 70 days, usually <20 days (thermotolerant coliforms and *Salmonella* spp.)
- Viruses 70 days, usually <100 days (enterovirus)
- Protozoa 150 days, usually <75 days (*Cryptosporidium* spp.)

5.1 BACTERIA

5.1.1 *Aeromonas* spp.

Aeromonads are associated with aquatic environments. Human infections are known to occur through ingestion of contaminated water or food (gastroenteritis), or via wound infection while swimming, although the evidence linking exposure and illness is not always robust (USFDA, 2012). *Aeromonas* spp. might be present in beach environments but there is insufficient data to indicate whether there is a risk to beach visitors. Only some species are known to cause illness in humans and the infective dose is not known.

Main reservoirs	Water, including estuarine and marine waters
Pathogenic strains	*A. hydrophila*, *A. caviae*, *A. dhakensis* and *A. veronii* are the predominant species isolated from humans with gastrointestinal disease (Fernández-Bravo and Figueras, 2020). Taxonomic changes within this genus has created confusion over virulence factors and whether there are differences between and within species (Rasmussen-Ivey *et al.*, 2016). *A. caviae* is isolated more often from clinical faecal samples, followed by *A. veronii*, *A. dhakensis* then *A. hydrophila* (Fernández-Bravo and Figueras, 2020).
Rate (NZ, per 100,000)	Unknown. Outbreaks are notifiable (no outbreaks were notified during 2019) (ESR Epidemiology Team, 2021).
Presence and survival in environment	Ubiquitous, waterborne bacteria (Tomás, 2012). Survives in soil and can multiply in wet soil (Brandi *et al.*, 1996; Vally *et al.*, 2004). Found in estuarine waters and sediments (Chaix *et al.*, 2017).
Presence in faeces	*A. caviae*, *A. sobria* and *A. hydrophila* were isolated from rectal swabs from sheep, cows and a horse (Ceylan *et al.*, 2009). Faecal carriage rates for animals have been reported in the range <1 to 7% (Igbinosa *et al.*, 2012).
Presence in beach sand	Found in the interstitial pore water of sand samples from freshwater beaches in Canada (Khan *et al.*, 2009).
Survival in beach sand	No relevant data located. Survival likely in interstitial pore water.

5.1.2 *Campylobacter* spp.

Campylobacter spp. are commonly found in animal faeces, particularly that of ruminants and poultry. Human illness (campylobacteriosis) arises through faecal-oral transmission from consuming contaminated food or water or being in contact with livestock.

Main reservoirs	Warm blooded animals
Pathogenic strains	*C. jejuni* is most frequently isolated from campylobacteriosis cases in New Zealand. *C. coli* also causes disease in New Zealand. Emerging species in humans are *C. concisus*, *C. upsaliensis*, *C. ureolyticus*, *C. hyointestinalis* and *C. sputorum* (Facciolà *et al.*, 2017).
Rate (NZ, per 100,000)	126.1 in 2019 (ESR Epidemiology Team, 2021)
Presence and survival in environment	A common zoonotic pathogen found in soil and water from faecal contamination. Has been detected in a range of freshwater environments in New Zealand including rivers and other surface waters, and shallow aquifers (Close *et al.*, 2008; Leonard *et al.*, 2020b; Leonard *et al.*, 2021; Phiri *et al.*, 2020b; Shrestha *et al.*, 2019).
Presence in faeces	Detected in the faeces of wild birds, livestock and pets in New Zealand (Anderson *et al.*, 2012; Irshad *et al.*, 2015; Mohan, 2015; Mohan *et al.*, 2013; Moriarty *et al.*, 2015; Moriarty *et al.*, 2011a; Moriarty *et al.*, 2011b; Moriarty *et al.*, 2008; Pattis *et al.*, 2017; Phiri *et al.*, 2020a; Rapp *et al.*, 2012).
Presence in beach sand	Detected in dry and wet sand samples from beaches in the UK, including beaches complying with FIB water standards (82/182, 45% positive) (Bolton *et al.*, 1999). Detected in sand samples from 13% (7/53) of beaches along the Californian coast (Yamahara *et al.*, 2012).
Survival in beach sand	The concentration of culturable *Campylobacter* spp. rapidly decreased in microcosm studies using marine beach sand seeded with primary treated sewage (Yamahara *et al.*, 2012). In freshwater beach environments, *Campylobacter* spp. might survive well in interstitial pore water (Whitman *et al.*, 2014). In microcosms using freshwater beach sand contaminated with sewage, the concentration of live *C. jejuni* decreased over 14 days, as measured by molecular methods (Eichmiller *et al.*, 2014).

5.1.3 Pathogenic *Escherichia coli*

As introduced in Section 3, *E. coli* are natural inhabitants of the gut of humans and other warm-blooded animals (particularly ruminants), so are excreted with faeces. However, some strains are known to be opportunistic human pathogens, causing enteric or extraintestinal disease. The main transmission route is faecal-oral, via consumption of contaminated food or water or contact with animals, particularly cattle. Pathogenic *E. coli* can be grouped into pathotypes or described using serotypes or virulence genes (Pakbin *et al.*, 2021). Strains of *E. coli* carrying the *stx* genes, the Shiga toxin-producing *E. coli* (STEC), are important

causes of intestinal and extraintestinal infections. The *E. coli* serotype O157:H7 is an example of an STEC since this serotype commonly carries one or more *stx* genes.

Main reservoirs	Ruminant animals, particularly cattle (Kim *et al.*, 2020).
Pathogenic strains	Diarrhoea and intestinal disorders are usually caused by *E. coli* belonging to seven pathotypes (Enteropathogenic *E. coli* (EPEC), Enterohaemorrhagic *E. coli* (EHEC), Enterotoxigenic *E. coli* (ETEC), Enteroinvasive *E. coli* (EIEC), Enteroaggregative *E. coli* (EAEC), Diffusely adherent *E. coli* (DAEC), or Adherent-Invasive *E. coli* (AIEC)) (Pakbin *et al.*, 2021). A suite of virulence factors is associated with each of these pathotypes (e.g. an STEC might be categorised as EHEC depending on the virulence genes present). Some of the genes encoding these virulence factors can be transferred between *E. coli*.
Rate (NZ, per 100,000)	STEC infection, 22.4 in 2019 (ESR Epidemiology Team, 2021).
Presence and survival in environment	Found in soil and water if introduced with faeces. Decay rate in water slower than enterococci, *Campylobacter* or *Salmonella* (Boehm *et al.*, 2018).
Presence in faeces	STEC have been detected in faeces from livestock in New Zealand (Cookson *et al.*, 2006; Irshad *et al.*, 2015; Irshad *et al.*, 2017; Moriarty *et al.*, 2011b; Moriarty *et al.*, 2008; Springer Browne *et al.*, 2018a; Springer Browne *et al.*, 2018b).
Presence in beach sand	*E. coli* O157 were not detected in 30 sand samples taken from a beach in Southern UK as part of an outbreak investigation (Harrison and Kinra, 2004). Viable *E. coli* were recovered from sand samples from a beach in Southern Florida but *E. coli* O157:H7 were not identified through PCR-based methods (Goodwin *et al.*, 2009). Other studies have used PCR-based methods to detect the presence of virulence genes among *E. coli* isolates or enrichments from freshwater beach sand (summarised in Whitman *et al*., 2014). The virulence marker gene *eae* was found but *stx* was not.
Survival in beach sand	*E. coli* O157:H7 survived for five days in sand in the presence of cattle faeces, both under dry conditions and with seawater tidal simulation (Williams *et al.*, 2007).

5.1.4 *Pseudomonas aeruginosa*

P. aeruginosa is an opportunistic pathogen, known for causing infections in immunocompromised patients and for antimicrobial resistance, so is mainly a concern in healthcare environments (Silby *et al.*, 2011). However, this pathogen is also widespread in the environment and known to cause skin and other soft tissue infections through recreational freshwater contact and through puncture wounds (Wilson and Pandey, 2022). As an indicator for freshwater quality, *P. aeruginosa* has been investigated as a novel health indicator for beach environments (Esiobu *et al.*, 2004).

Main reservoirs	Freshwater, animals, humans
Pathogenic strains	Although there are a range of genes known to be important for pathogenicity, the species is known to rapidly mutate and adapt so all strains are considered potentially pathogenic (Qin *et al.*, 2022; Silby *et al.*, 2011)
Rate (NZ, per 100,000)	Unknown. Outbreaks are notifiable (no outbreaks were notified during 2019) (ESR Epidemiology Team, 2021).
Presence and survival in environment	Has been detected in ground water and surface waters (Wei *et al.*, 2020). In New Zealand, genetic material suggesting the presence of bacteria from the *Pseudomonas* genus was detected in groundwater samples (Sirisena *et al.*, 2018). Survives well in soil and water (EFSA Panel on Animal Health and Welfare *et al.*, 2022).
Presence in faeces	Can be present in the faeces of healthy humans (Estepa *et al.*, 2014). Might be shed into the environment by dogs and cats through their faeces (and urine and saliva) but data are scarce (EFSA Panel on Animal Health and Welfare *et al.*, 2022; Płókarz and Rypuła, 2022).
Presence in beach sand	*P. aeruginosa* were detected in sand samples from marine beaches in Sao Paulo (Brazil) (Sanchez *et al.*, 1986; Whitman *et al.*, 2014). *P. aeruginosa* were incidentally detected in sand samples from beaches in temperate South Carolina (Stevens *et al.*, 2012). In Japan, *Pseudomonas* spp. were detected through PCR-based methods in sand samples taken after a typhoon event, at different depths (Suzuki *et al.*, 2018). No further speciation was done to identify whether *P. aeruginosa* were present. *P. aeruginosa* has been detected in other studies of beach sand from beaches in subtropical regions, sometimes at high concentrations (10^7 cells/g sand) (Whitman *et al.*, 2014).
Survival in beach sand	No relevant data located.

5.1.5 *Salmonella* spp.

The group *Salmonella enterica* subsp. *enterica* contains multiple serotypes, many of which are important causes of human disease through faecal-oral transmission. All serotypes are considered to be potential human pathogens although a few serotypes are commonly isolated from human clinical samples. Humans are the only hosts for serotypes Typhi and Paratyphi, which cause enteric fever (typhoid or paratyphoid fever) but can also be shed by asymptomatic carriers. Typhi and Paratyphi are not endemic in New Zealand but have caused infections in people returning from countries where the disease is endemic, thus there is a risk of these being introduced into the environment with human faeces. The remainder of this section focuses on the non-typhoidal salmonellae serotypes, which can be present in the faeces of a range of warm- and cold-blooded animals and humans. The disease, salmonellosis, can occur through consuming contaminated food or water, or being in contact with animals. Animals can also become ill from a *Salmonella* infection, which increases faecal shedding.

Main reservoirs	Warm- and cold-blooded animals, humans
Pathogenic strains	All *Salmonella* spp. considered potentially pathogenic. The *Salmonella* serotypes Typhimurium, Enteritidis and Bovismorbificans are most often isolated from infected humans in New Zealand (ESR Enteric Reference Laboratory, 2022).
Rate (NZ, per 100,000)	24.2 in 2019 (ESR Epidemiology Team, 2021).
Presence and survival in environment	Found in soil and water if introduced with faeces, with variable survival times. Has been detected in surface waters in New Zealand (Leonard *et al.*, 2020b; Leonard *et al.*, 2021; Till *et al.*, 2008).
Presence in faeces	New Zealand studies indicate *Salmonella* spp. Are more likely to be found in faecal samples from young or sick animals (Al Mawly *et al.*, 2015a; Lawrence *et al.*, 2019; Moriarty *et al.*, 2011a; Moriarty *et al.*, 2011b; Moriarty *et al.*, 2008; Stevenson *et al.*, 2016). Survival highly variable but several months possible under field conditions (Ongeng *et al.*, 2013).
Presence in beach sand	Detected in sand samples from 11% (6/53) of beaches along the Californian coast (Yamahara *et al.*, 2012). Detected in dry and wet sand samples from beaches in the UK, including beaches complying with FIB water standards (10/182, 6% positive) (Bolton *et al.*, 1999). Not detected in sand samples from marine beaches in Sao Paulo (Brazil) (Sanchez *et al.*, 1986; Whitman *et al.*, 2014).
Survival in beach sand	The concentration of culturable *Salmonella* spp. Decreased in microcosm studies using marine beach sand seeded with primary treated sewage (Yamahara *et al.*, 2012). In microcosms using freshwater beach sand contaminated with sewage, the concentration of live *Salmonella* Typhimurium decreased over 14 days, as measured by molecular methods (Eichmiller *et al.*, 2014).

5.1.6 *Staphylococcus aureus*

S. aureus are common inhabitants of human skin and mucous membranes, being persistently carried by an estimated 20–30% of people, and intermittently for 60% of people (Argudín *et al.*, 2010). In a New Zealand study, repeat nasal swabs of 70 children identified 30 (45%) who carried *S. aureus* consistently (Scott *et al.*, 2018). *S. aureus* are also present in raw sewage (Kozajda and Jeżak, 2020). These bacteria are important human pathogens and cause a range of soft tissue, enteric and invasive conditions. The incidence of methicillin-resistant *S. aureus* (MRSA) infections continues to increase and more community-associated cases are being reported (Plano *et al.*, 2013).

S*. aureus* is a well-known cause of food poisoning, which occurs when these bacteria are able to multiply in food and produce an enterotoxin. However, in beach environments the main exposure route presenting a risk of infection is skin contact with *S. aureus* in the sand. This can lead to skin and soft tissue infections. The most likely sources of *S. aureus* in the beach environment are raw sewage contamination or shedding from the skin of beach

visitors. Regarding the latter, the *S. aureus* concentrations in seawater and sand have been positively associated with the density of beach visitors, although not consistently (Esiobu *et al.*, 2013; Papadakis *et al.*, 1997; Topić *et al.*, 2021). Researchers undertaking surveys of *S. aureus* in sand from subtropical beaches in California (USA) proposed that the concentrations (187 CFU *S. aureus*/100 g dry sand; 95% CI 98-390) were not high enough to cause skin/wound infections, but admitted more accurate assessment was needed (Goodwin *et al.*, 2012). Much higher concentrations have been reported elsewhere (Esiobu *et al.*, 2013). It has been suggested that *S. aureus* could be a useful indicator for non-enteric infections (Mohammed *et al.*, 2012).

Main reservoirs	Humans, animals (nose, throat, skin)
Pathogenic strains	All isolates are considered potentially pathogenic. Strains (clonal lineages) of *S. aureus* can be host specific, but some strains can colonise multiple hosts (Fitzgerald, 2012). Changes in host range can occur (Smith, 2015).
Rate (NZ, per 100,000)	Unknown. Outbreaks are notifiable as are cases of staphylococcal intoxication (no outbreaks or cases were notified during 2019) (ESR Epidemiology Team, 2021).
Presence and survival in environment	Widespread in the environment, including water (USFDA, 2012). Has been detected in soils from New Zealand dairy farms (Amoafo *et al.*, 2020).
Presence in faeces	*S. aureus* has been detected in animal faeces (Friese *et al.*, 2013; He *et al.*, 2013). Has been detected in faeces from birds (including seagulls) and dogs (Topić *et al.*, 2021).
Presence in beach sand	Detected in sand samples from 14% (5/37) of beaches along the Californian coast (one beach was MRSA positive) (Yamahara *et al.*, 2012). Also detected in samples of wet sand taken from freshwater beaches in Ohio (USA), with an overall prevalence of 20.5% (43/210) (Thapaliya *et al.*, 2017). MRSA were detected in 15/210 samples and methicillin-susceptible *S. aureus* (MSSA) were detected in 28. Detected in samples of dry and swash zone sand from a beach in Florida, but not in subtidal sand (MRSA detected in the dry samples only) (Plano *et al.*, 2013).
Survival in beach sand	*S. aureus* is likely to survive quite well in beach sand, being tolerant of high temperatures, and dry and salty conditions (Topić et al., 2021). A study in Florida (noting this is a subtropical region) found higher concentrations of *S. aureus* in dry (supratidal) sand samples compared to wet (intertidal) and inundated (subtidal) samples (Shah et al., 2011). Another study in Florida found that approximately half of the recovered *S. aureus* population were attached to sand grains rather than existing in interstitial spaces, in both wet and dry sand samples (Esiobu et al., 2013). It is not clear how quickly *S. aureus* might attach to sand grains, nor how this influences their survival in the beach environment, although it is likely to support longer persistence. This study also found highest concentrations 4-8 cm deep, where the *S. aureus* was

	protected from UV radiation and provided with sufficient moisture and nutrients to survive. Controlled studies using microcosms suggest that *S. aureus* can grow well in beach sand in intertidal zones, although growth might be limited by natural predation (Mohammed *et al.*, 2012). In microcosms using freshwater beach sand contaminated with sewage, the concentration of a live MRSA strain decreased over 14 days, as measured by molecular methods (Eichmiller *et al.*, 2014). Surveys of beach sands in California and Ohio found higher numbers of *S. aureus* during periods of warmer temperatures (Goodwin *et al.*, 2012; Thapaliya *et al.*, 2017). These data suggest that warmer temperatures support survival and/or permitted multiplication, however warmer temperatures also attract more visitors to beach areas, who might be introducing *S. aureus* (see above).

5.1.7 *Vibrio* spp.

The three main species important for human health are *Vibrio parahaemolyticus* (primarily causes foodborne disease via seafood consumption), *Vibrio vulnificus* (a cause of foodborne disease but also of serious wound infections) and *Vibrio cholerae* (in New Zealand, where cholera is not endemic, serotypes other than O1 and O139 have caused disease in non-travellers, suggesting domestic exposure is occurring) (Baker-Austin *et al.*, 2018; Powell *et al.*, 2019).

As natural inhabitants of the aquatic environment, these bacteria are important hazards for seafood consumers and swimmers. However, there is some evidence they can be present in wet sand, where they could infect wounds of beach visitors. Such infections can lead to secondary septicaemia (Baker-Austin *et al.*, 2018). Other species, such as *Vibrio alginolyticus* and *Vibrio hollisae* are also known to cause wound infections (Baker-Austin *et al.*, 2018).

Because *Vibrio* spp. thrive under warmer conditions, *Vibrio* infections are more common in tropical and subtropical locations compared to temperate locations and there are more sand-related data from these warmer locations. For example, several studies in Israel have found *V. alginolyticus*, *V. parahaemolyticus* and *V. vulnificus* in wet sand samples (summarised by Whitman *et al.*, 2014). *V. vulnificus* was detected in sand from subtropical beaches in Florida, including dry sand samples (Abdelzaher *et al.*, 2010; Shah *et al.*, 2011). The total *Vibrio* spp. count in samples of both backshore and foreshore sand from beaches in Hawaii was as high as 10^4 CFU/100 g (Cui et al., 2013). *Vibrio* spp. were also among the genera colonising plastic fragments (microplastics) collected from tropical Singapore beaches (Curren and Leong, 2019).

Main reservoirs	Water
Pathogenic strains	The *Vibrio* species *parahaemolyticus*, *vulnificus* and *cholerae* are important human pathogens but some strains within each species are more likely to be detected in human clinical samples.
Rate (NZ, per 100,000)	Unknown. Outbreaks are notifiable and one outbreak was notified during 2019 (ESR Epidemiology Team, 2021). Cases

	of *V. parahaemolyticus* infection should also be notified under acute gastroenteritis (49 cases were notified during 2019).
Presence and survival in environment	Naturally present in freshwater and coastal marine environments. Can be free living but more likely to colonise fish and marine organisms or attach to biotic or abiotic surfaces including plankton and sediments (Baker-Austin *et al.*, 2018).
Presence in faeces	Present in the faeces of infected humans.
Presence in beach sand	A study of a surf beach in Japan after a typhoon detected *Vibrio* spp. in sand collected from the water's edge using molecular techniques, but did not detect *Vibrio* spp. in landward sand samples (Suzuki et al., 2018).
Survival in beach sand	No relevant data located.

5.1.8 *Yersinia* spp.

In New Zealand, *Yersinia enterocolitica* and *Yersinia pseudotuberculosis* are important causes of foodborne gastroenteritis (yersiniosis). None of the literature reviewed suggested that *Yersinia* spp. should be considered as potential pathogens in beach environments. However, these bacteria share similar characteristics to others included in this section, in that they are carried by animals and, via direct faecal contamination or faecal-contaminated water, could enter beach environments. Thus, they have been considered.

Main reservoirs	Animals
Pathogenic strains	*Y. enterocolitica* is most commonly isolated from yersiniosis cases in New Zealand but *Y. pseudotuberculosis* is also recovered (diagnostic protocols means this latter species is likely to be underreported). The most common *Y. enterocolitica* types recovered from clinical samples during 2019 were biotype 2/3 serotype O:9, biotype 1A and biotype 4 serotype O:3 (ESR Epidemiology Team, 2021).
Rate (NZ, per 100,000)	24.1 in 2019 (ESR Epidemiology Team, 2021).
Presence and survival in environment	Found in soil and water if introduced with faeces (Sutherland *et al.*, 2009).
Presence in faeces	Present in animal faeces, including healthy livestock and companion animals. Has been detected in faeces from infected goats and healthy deer in New Zealand, and from cattle tested at veterinary diagnostic laboratories (Lanada *et al.*, 2005; Lawrence *et al.*, 2019; Pattis *et al.*, 2017).
Presence in beach sand	No relevant data located.
Survival in beach sand	No relevant data located.

5.2 VIRUSES

A range of enteric viruses circulate among humans and might enter beach environments with human faecal contamination, directly or via water inflows. Viruses can be infective in small doses and can remain viable in the environment for weeks. Non-enveloped viruses tend to be more environmentally stable because these do not rely on an outer lipid membrane for their survival. Disinfection of wastewater is not common practice in New Zealand and wastewater treatment processes may not be effective for virus inactivation, although UV radiation reduces viral concentrations in detention ponds (Natural Resource Management Ministerial Council *et al.*, 2006). As a result, these viruses can be present and persist in receiving waters (Greening, 2006). To be a faecal-borne hazard, the viruses must be infective in faeces.

Rather than list all potential viruses, four examples are considered based on their use in environmental health risk assessment literature and their survival characteristics: Adenovirus, which is a widely used indicator of human faecal contamination, hepatitis A virus and human norovirus which can be found in the environment as a result of human faecal contamination, and rotavirus as an example of a potentially zoonotic virus.

Molecular methods are used to detect norovirus in clinical and environmental samples. These do not indicate the presence of viable viruses. Data on the presence and survival of viruses in beach sand are scarce so some information from subtropical and tropical locations has been included.

5.2.1 Human adenovirus (hAdV)

Adenoviruses are non-enveloped, double-stranded DNA viruses, which makes them very hardy in the environment. Adenoviruses can infect multiple hosts or be host-specific. Strains within the genus *Mastadenovirus*, which infect mammals, are identified by serotyping (traditional) and/or genotyping (increasingly common). The group causing human infections, called human adenovirus (hAdV), is used as an indicator of human faecal contamination (Hewitt *et al.*, 2013). Most infections are asymptomatic or mild but can progress to severe illness with a range of disease presentations including respiratory, ocular and gastrointestinal symptoms. The virus can be spread by inhalation of aerosolised droplets, via fomites and along the faecal-oral transmission pathway (Khanal *et al.*, 2018). Latent infections can occur.

Main reservoirs	Humans
Pathogenic strains	There are over 100 hAdV genotypes described (Human Adenovirus Working Group, 2023). They are grouped into seven species (hAdV-A to hAdV-G). Species hAdV-F (types HAdV-F40 and HAdV-F41) is common among children with gastroenteritis (Rafie *et al.*, 2021). Adenoviruses can cross species barriers (Borkenhagen *et al.*, 2019).
Rate (NZ, per 100,000)	Unknown. Outbreaks are notifiable and two outbreaks were notified during 2019 (ESR Epidemiology Team, 2021).
Presence and survival in environment	Found in soil and water if introduced with human faeces. HAdV has been detected in treated effluent wastewater, river water, urban stream water and estuarine water in New Zealand (Hewitt *et al.*, 2013).
Presence in faeces	Concentrations in human faeces can reach 10^{11} particles/g (Hewitt *et al.*, 2013; Wold and Horwitz, 2007).

Presence in beach sand	Detected in sand samples from Portugal (Monteiro *et al.*, 2016). Not detected in sand samples from tropical/dry beaches in Southern Florida, USA (Goodwin *et al.*, 2009).
Survival in beach sand	No relevant data located.

5.2.2 Hepatitis A virus (HAV)

HAVs are single-stranded RNA viruses that are quasi-enveloped while in the host but are non-enveloped when excreted with faeces (Migueres *et al.*, 2021). Humans are the only natural host for HAV, which is transmitted between people along a faecal-oral pathway, usually through contaminated water or food. Overseas travel is a common risk factor reported by cases of HAV infection in New Zealand.

Main reservoirs	Humans
Pathogenic strains	One serotype and six genotypes in the genus *Hepatovirus*, within the family *Picornaviridae* (Greening and Cannon, 2016). Only genotypes I (a and b subtypes), II (a and b subtypes) and III have been associated with human infections.
Rate (NZ, per 100,000)	1.2 in 2019 (ESR Epidemiology Team, 2021).
Presence and survival in environment	Found in soil and water if introduced with human faeces. Can persist for weeks in water, especially at low temperatures (Cook *et al.*, 2018).
Presence in faeces	Viruses are usually shed at the highest concentrations (>10^6 viral particles/g faeces) in faeces 2–3 weeks prior to clinical symptoms but are also excreted several months after infection (Greening and Cannon, 2016). Virus concentrations excreted from asymptomatic/subclinical cases are as high as for symptomatic individuals, which is important since most HAV infections are asymptomatic or subclinical and occur in young children (Costafreda *et al.*, 2006; Hollinger and Emerson, 2007). HAV infection is more likely to be symptomatic for adults.
Presence in beach sand	Detected in sand samples from Portugal (Monteiro *et al.*, 2016). Not detected in wet and dry sand samples from a subtropical beach in Florida, USA (Abdelzaher *et al.*, 2010).
Survival in beach sand	No relevant data located.

5.2.3 Human norovirus (hNoV)

Noroviruses are non-enveloped, single-stranded RNA viruses than infect a range of mammalian species. They are genetically diverse and their classification is subject to change. A recent review proposed 10 genogroups (GI-GX) and 49 genotypes (Chhabra *et al.*, 2019). Noroviruses that infect humans tend to cluster into three genogroups, so are referred to as human noroviruses. These cause gastrointestinal infection, resulting in viruses being shed with vomitus and faeces, with higher viral concentrations in the latter (Robilotti *et al.*, 2015).

Main reservoirs	Humans
Pathogenic strains	Genetically diverse. Noroviruses that infect humans (hNoV) belong to three genogroups (GI, GII and GIV). GII is the predominant genogroup associated with gastroenteritis outbreaks in New Zealand and is found elsewhere in the world (Greening *et al.*, 2012; Lim *et al.*, 2016a; Pattis *et al.*, 2019; van Beek *et al.*, 2013).
Rate (NZ, per 100,000)	Unknown. Outbreaks are notifiable and 180 outbreaks were notified during 2019 (ESR Epidemiology Team, 2021). Cases might also be notified under acute gastroenteritis (20 cases were notified during 2019).
Presence and survival in environment	Found in soil and water if introduced with human faeces. Has been detected in New Zealand rivers, urban streams, and estuarine waters (Hewitt *et al.*, 2013; Williamson *et al.*, 2011).
Presence in faeces	Concentrations in human faeces vary but typically are 10^8 viral particles/g but can be up to 10^{11} particles/g (Atmar *et al.*, 2008). Faecal excretion of norovirus from asymptomatic cases is common, particularly among children (Robilotti *et al.*, 2015).
Presence in beach sand	Not detected in wet and dry sand samples from a subtropical beach in Florida, USA, nor in sand samples from Portugal (Abdelzaher *et al.*, 2010; Monteiro *et al.*, 2016).
Survival in beach sand	No relevant data located.

5.2.4 Rotavirus

Rotaviruses are non-enveloped double-stranded RNA viruses that are highly diverse and infect a range of animals. Although animal and human strains are usually distinct, cross-species transmission can occur when strains are closely related or have undergone natural genetic reassortment (Greening and Cannon, 2016). Globally, rotavirus is a leading cause of severe, acute dehydrating diarrhoea among infants and young children (Omatola and Olaniran, 2022). Transmission follows the faecal-oral route, usually via contaminated food or water. A vaccine is now included in the New Zealand childhood immunisation schedule.

Main reservoirs	Animals, humans
Pathogenic strains	There are ten species (A-J) of which A, and less commonly B, C and H, infect humans (Crawford *et al.*, 2017; Greening and Cannon, 2016). Group A, B, C and H also infect other animals especially young domestic and farm animals including birds.
Rate (NZ, per 100,000)	Unknown. Outbreaks are notifiable and one outbreak was notified during 2019 (ESR Epidemiology Team, 2021). Cases might also be notified under acute gastroenteritis (1 case was notified during 2019).
Presence and survival in environment	Found in soil and water if introduced with faeces. Highly stable in the environment and can remain infectious for weeks at 4°C

	and 20°C in environmental waters (Greening, 2006). Detected in two New Zealand rivers (Williamson *et al.*, 2011).
Presence in faeces	Detected faecal samples from New Zealand calves (Al Mawly *et al.*, 2015b). Up to 10^{10} virus particles/g faeces can be shed by humans prior to the onset of symptoms and for up to a month after the onset of symptoms (Pickering *et al.*, 1988).
Presence in beach sand	No relevant data located.
Survival in beach sand	No relevant data located.

5.3 PARASITES

Parasites, by their nature, rely on a host to complete their full lifecycle and some are shed into the environment with faeces. The focus of this review is on protozoan parasites, and experts have agreed that there are three species of importance for sand safety: *Cryptosporidium* spp., *Giardia duodenalis* and *Toxoplasma gondii* (Sabino *et al.*, 2014).[6] These protozoan parasites cause human disease in New Zealand and have environmentally persistent life stages (oocysts, cysts) that remain viable in water and soil for several weeks or longer.

However, the potential for sporadic human infection due to metazoan parasites in beach sand is also noted, particularly from the helminths and nematodes which might infect humans via the gastrointestinal tract or skin. It has been proposed that metazoan parasites could be additional indicators for beach hygiene (Manjarrez *et al.*, 2019). For example, the helminth *Toxocara* spp., a common parasitic roundworm of domesticated animals, has been of interest because their worldwide distribution (Sabino *et al.*, 2014). *Toxocara canis* is a common parasite of dogs and can be introduced into beach environments with dog faeces. Beach sand surveys have detected *Toxocara* spp. and other parasites (Bojar and Kłapeć, 2018; Ramos *et al.*, 2020). The feline hookworm in the species *Anclyostoma* has caused an outbreak in a beach setting (WHO, 2021).

5.3.1 *Cryptosporidium* spp.

The most common species that cause human infection are the zoonotic species *Cryptosporidium parvum*, and the human-associated species *Cryptosporidium hominis* (Vanathy *et al.*, 2017). Molecular techniques are used to subdivide strains within these species into subtypes. Infection is via the faecal-oral route. Humans are the primary host for *C. hominis*.

Ingested as an environmentally-stable oocyst, *Cryptosporidium* spp. passes through several life-cycle stages within a single host to form new oocysts, which are excreted with faeces (Vanathy *et al.*, 2017). Both thin-walled and thick-walled oocysts are formed but it is the latter that survives well in the environment so is the primary cause of disease. Outbreaks of cryptosporidiosis are often associated with contaminated water.

Main reservoirs	Water (oocysts), warm blooded animals (*C. parvum*), humans (*C. hominis*)
Pathogenic strains	Several *Cryptosporidium* species have been isolated from humans but *C. parvum* is the most frequently reported

[6] In this document, *Giardia lamblia* is referred to by the more accepted synonym *Giardia duodenalis* (also synonymous with *Giardia intestinalis*).

	zoonotic species (Erickson and Ortega, 2006; Garcia-R *et al.*, 2020; USFDA, 2012). Humans are the primary host for *C. hominis* (EFSA Panel on Biological Hazards *et al.*, 2018).
Rate (NZ, per 100,000)	21.0 in 2019 (ESR Epidemiology Team, 2021)
Presence and survival in environment	Found in soil and water if introduced with faeces, can survive many months (Erickson and Ortega, 2006). Oocysts have been detected in New Zealand surface waters (Leonard *et al.*, 2020b; Leonard *et al.*, 2021; Phiri *et al.*, 2020b; Till *et al.*, 2008).
Presence in faeces	Detected in faeces from livestock and wild birds in New Zealand (Moriarty *et al.*, 2011a; Moriarty *et al.*, 2011b; Moriarty *et al.*, 2008).
Presence in beach sand	*Cryptosporidium* spp. were detected by PCR, but not microscopy, in wet and dry sand samples from a subtropical beach in Florida, USA (Abdelzaher *et al.*, 2010). In another Florida study, *Cryptosporidium* spp. were detected by microscopy in one sample of wet (intertidal) sand, at a low concentration (0.63 oocysts per 100 g sand) (Shah *et al.*, 2011). This study analysed 12 samples from each dry, wet and inundated (subtidal) beach zone.
Survival in beach sand	No studies located.

5.3.2 *Giardia duodenalis*

G. duodenalis is a zoonotic pathogen spread via faecal-oral transmission. Like *Cryptosporidium* spp., *G. duodenalis* completes its life-cycle within a single host, from ingestion of the environmentally-stable cyst through to excretion of new cysts with faeces (Adam, 2001). In higher income countries giardiasis is associated with consumption of contaminated water or food and direct contact with faeces (e.g. through changing nappies) (Krumrie *et al.*, 2022).

Main reservoirs	Humans, animals, water (cysts)
Pathogenic strains	*G. duodenalis* infects humans and mammals (Feng and Xiao, 2011). There are eight major genetic groups (assemblages), but only two (A and B) are capable of causing disease in humans (Winkworth, 2010).
Rate (NZ, per 100,000)	All giardiasis cases, 35.6 in 2019 (ESR Epidemiology Team, 2021)
Presence and survival in environment	Found in soil and water if introduced with faeces. Can remain infective in soil for over a month at low temperatures, but infectivity is not well sustained at higher temperatures (Feng and Xiao, 2011). Cysts have been detected in New Zealand surface waters (Leonard *et al.*, 2020b; Leonard *et al.*, 2021; Phiri *et al.*, 2020b; Till *et al.*, 2008).

Presence in faeces	Detected in livestock faeces in New Zealand (Abeywardena *et al.*, 2012; Moriarty *et al.*, 2011b; Moriarty *et al.*, 2008).
Presence in beach sand	*Giardia* spp. were not detected by PCR or microscopy in wet and dry sand samples from subtropical beaches in Florida, USA (Abdelzaher *et al.*, 2010; Shah *et al.*, 2011). *Giardia duodenalis* was detected in 2/96 dry sand samples taken from beaches in São Paulo (Brazil) during spring and summer months (Zanoli Sato *et al.*, 2005).
Survival in beach sand	No studies located.

5.3.3 *Toxoplasma gondii*

T. gondii is excreted by the definitive host, cats, and develops into an environmentally stable oocyst form. Toxoplasmosis appearing among marine vertebrates demonstrates that these oocysts migrate into the marine environment (Roberts *et al.*, 2021). Human toxoplasmosis outbreaks have occurred but these are more often food or waterborne (Pinto-Ferreira *et al.*, 2019). Because most human infections are asymptomatic, toxoplasmosis cases tend to appear sporadically, with pregnant women being a high-risk population.

The risk from beach sand is elevated in areas with high cat populations.

Main reservoirs	Soil (oocysts), cats
Pathogenic strains	Most virulence studies have involved genotypes I, II and III, but there is very little known about the virulence of each of these in humans (FSANZ, 2013). Present in the environment as oocysts (sporozoites).
Rate (NZ, per 100,000)	Unknown. Not notifiable unless there is an outbreak.
Presence and survival in environment	Detected in soils from rural and urban locations, more often when cats are present and if conditions are moist (Shapiro *et al.*, 2019). Can contaminate water. Oocysts can survive for many months and even years in moist soil or sand, or water (Innes, 2010; Shapiro *et al.*, 2019; Torrey and Yolken, 2013).
Presence in faeces	Only excreted in the faeces of cats, the definitive host (Innes, 2010).
Presence in beach sand	No relevant data located.
Survival in beach sand	No relevant data located. Likely to survive well.

5.4 FUNGI

Fungi are environmentally widespread and human infections are often opportunistic. Infections can become very serious for immunocompromised individuals. On the beach, infections could result from direct contact with fungal pathogens in the sand or inhalation of fungal spores in the beach environment. Inhaled fungal spores, such as those of the genera *Alternaria* and *Cladosporium*, can also cause allergic responses and related conditions such as asthma (Whitman *et al.*, 2014). However, these are more likely to cause problems in enclosed spaces rather than beaches. Potentially pathogenic fungi, such as *Fusarium* spp.

and *Aspergillus* spp., could be part of the natural biological community at beaches (Abreu *et al.*, 2016). Beach wrack (organic material) can also harbour fungi (Solo-Gabriele *et al.*, 2016). Others fungal groups, such as yeasts (e.g. *Candida* spp.) and dermatophytes, are more likely to be associated with the presence of people at the beach (Abreu *et al.*, 2016). This is one reason for FIB being a poor indicator for pathogenic fungi, although one study did find correlations between enterococci and yeast concentrations in beach sand (Shah *et al.*, 2011).

A variety of fungal genera have been detected in beach sand (Solo-Gabriele *et al.*, 2016; Whitman *et al.*, 2014). People visiting beaches influence the abundance and diversity of sand fungal species. A study of nine beaches in temperate South Carolina (USA) found a positive correlation between the concentration of fungi in sand samples and the number of people present in the sampling area (Stevens *et al.*, 2012). Other studies have found similar correlations (Papadakis *et al.*, 1997; Vogel *et al.*, 2007).

The WHO lists eight fungal groups that could be important for sand safety (WHO, 2021): *Aspergillus* spp., *Cryptococcus* spp., *Histoplasma capsulatum*, *Blastomyces dermatitidis*, *Fusarium* spp., *Cladophialophora bantiana*, *Candida* spp. and the dermatophytes. Of these, infection through inhalation is important for the first five listed and not all will be important for New Zealand. For example, *H. capsulatum*, causing histoplasmosis, is not endemic in New Zealand although data are scarce (Chakrabarti and Slavin, 2011). On the other hand, *Cryptococcus* spp., causing cryptococcosis, was initially associated with tropical and subtropical climates (Sabino *et al.*, 2014) but this yeast has now been found in temperate countries including New Zealand (Springer and Chaturvedi, 2010). One species, *Cryptococcus neoformans*, has been identified as a critical priority fungal pathogen because of its ability to cause invasive, drug-resistant infections (WHO, 2022).

Of those remaining from the WHO list above, *C. bantiana* causes a severe, but very rare, disease affecting the brain (Kantarcioglu *et al.*, 2017). *Candida* spp. are opportunistic pathogens frequently found in sand (Solo-Gabriele *et al.*, 2016). One species, *Candida albicans*, is a human gastrointestinal tract commensal so the presence of this species in the environment indicates contamination with human faeces. *C. albicans* and *Candida auris* have both been identified as critical priority fungal pathogens by the WHO, with antifungal drug resistance being of great concern (WHO, 2022). The dermatophytes are moulds that cause superficial infections of the skin, hair or nails, e.g. *Trichophyton* spp. and *Microsporum* spp. (Solo-Gabriele *et al.*, 2016).

The fungal species found in seawater, beach sand and human clinical samples have been reviewed (Solo-Gabriele *et al.*, 2016). This created a list of 26 fungal species found in both beach sand and human clinical samples. When additionally considering a study of invasive mycotic infections in the San Francisco Bay area during the 1990s, experts have determined that *Aspergillus*, *Candida*, *Fusarium* and dermatophytes like *Microsporum* and *Trichophyton* represent the majority of fungal isolates from clinical samples important for human health (Rees *et al.*, 1998; Solo-Gabriele *et al.*, 2016).

The above shows that it is difficult to determine which fungal species could pose the greatest risk to beachgoers. An expert group has concluded that the total fungal colony count, plus *C. albicans* and dermatophytes, were useful health indicators for beach sand (Sabino *et al.*, 2014). However, they acknowledged that there was a lack of data to underpin risk-based decision making, such as dose response and epidemiological data. Cases can be sporadic and symptoms can be delayed, making it difficult to associate beach sand exposure with illness (Weiskerger and Brandão, 2020). Thus the question remains: At what level does the presence of these fungi in sand pose a risk to human health?

Furthermore, it has been proposed that fungal speciation, rather than total fungal colony count, is important because this can help identify sources of contamination (Solo-Gabriele *et*

al., 2016). Such methods might include testing for fungi that are not important human pathogens but indicate the potential presence of other pathogenic microorganisms.

The Mycosands Initiative was a multi-country project to generate data on fungi in beach sands and waters of both coastal and freshwater bathing sites (Brandão *et al.*, 2021). Together, 372 dry sand samples were taken from 91 beaches in 13 countries, including three beaches in Sydney, Australia. The median concentration of culturable fungi in sand was 89 CFU/g and the maximum 6,400 CFU/g. Fungi concentrations in sand were higher than water samples (median 0 CFU/ml, maximum 1,592 CFU/ml), although these results are not directly comparable. It was also found that the fungi concentrations were:

- Higher in inland freshwater beach sands where salinity was lower and the organic load higher, although there were fewer samples compared to coastal beach sands. Note that the wave energy on a beach is likely to influence fungal concentrations as has been found for bacteria (see Section 3.1.2).
- Not significantly different between urban and non-urban beaches although the species present tended to reflect the environment, with human-associated species more likely to be found in urban beaches. Note that other studies have found correlations between beach types and usage, and fungal concentrations (Stevens *et al.*, 2012; Vogel *et al.*, 2007).
- Significantly higher in samples taken during autumn/winter compared with spring/summer. Elevated temperatures become inhibitory to many fungi, and exposure to UV contributes to their death (statistically significant, negative correlations were found between the hours of sunshine on the sampling day and the concentrations of total fungi, *Aspergillus* spp. and *Candida* spp.). Note that the depth of the sand sample is important and some sampling approaches avoid surface sand, instead taking sand samples a few centimetres below the surface (Tugrul-Icemer and Topaloglu, 2011).

Overall, by comparing the geographical regions and individual beaches, the authors found that the presence and composition of fungal microbiota is site-dependant, although *Rhodotorula* and *Candida* species were ubiquitous. The genera most frequently found were *Aspergillus* spp., *Candida* spp., *Fusarium* spp. and *Cryptococcus* spp.

One study, which formed part of the Mycosands Initiative, focused on samples from a regularly groomed, artificial sand beach in Slovenia (Novak Babič *et al.*, 2022). The researchers found that the mycobiota of the sand and water were different, and that the diversity of the fungi present in sand could be related to environmental factors. One finding of relevance to this review is that during the official bathing season, when beach visitor numbers were high, four fungal genera were present more consistently and/or at higher concentrations. These genera were *Actinomucor*, *Condenascus Stachybotrys* and *Meyerozyma*. Of these, *Meyerozyma* spp. was proposed as a candidate for indicating "human pollution", although this study did not assess correlations between any of these proposed indicator genera and fungi known to be important for human infection (e.g. *Aspergillus* spp., *Candida* spp.).

6. MICROBIAL HAZARDS IN NZ SAND

Summary

Beach sands have been tested for enterococci in the Wellington and Auckland regions. Samples from two beaches in the Wellington area contained enterococci at concentrations that were not detectable or ≤30 CFU/g. Surveys of seven beaches in the Auckland area identified that enterococci were widespread but elevated concentrations were associated with decaying seaweed. Enterococci concentrations in seaweed and sand samples were typically in the range 10^3-10^4 CFU/g dry weight.

Samples of liquefaction sediment collected one month after the 22 February 2011 Canterbury earthquake contained *E. coli* at concentrations >200 MPN/g dry weight. Concentrations decreased over subsequent weeks. Field trials of sewage-amended silt found that *C. perfringens* spores and the genetic material from enteric viruses persisted for five months. Enterococci have also been detected in sediments collected from New Zealand streams and rivers, at variable concentrations.

The effects of climate change on coastal areas are likely to affect sand microbiological communities and alter the risk to humans, but not all changes will be favourable for pathogenic microorganisms. Any change in risk will depend on patterns at a local level, with alterations in temperature, solar irradiation, precipitation, sea level, wave activity and human behaviour all interacting.

6.1 PRESENCE IN SAND: NZ CASE STUDIES

No studies of microbiological pathogens in New Zealand beach sands were located. Two studies of enterococci in beach sands are described below.

Greater Wellington Regional Council has tested beach sediment samples for *Enterococci*. Samples were taken from two locations during winter (June, 2022) and tested by a commercial laboratory. The samples were not taken in response to a contamination event but as part of an investigation to assess *Enterococci* concentrations in beach substrate. Seven samples were taken from Owhiro Bay, where the beach substrate is stones, gravel and coarse sand, and seven from the finer sands of Scorching Bay. *Enterococci* were not detected (<10 CFU/g) in ten samples. The concentration of *Enterococci* was ≤30 CFU/g in the three positive samples from Owhiro Bay and the one positive sample from Scorching Bay.

During the 1990s, an investigation of the environmental abundance and distribution of enterococci included surveys of seven beaches of the Auckland region, with the support of Auckland Regional Council (Anderson, 1999). During 1995, four beaches were sampled as part of a wider investigation of enterococci abundance in the environment, these being St Mary's Beach, Blockhouse Bay, Wenderholm Beach and Mission Bay. Samples of beach sand, seaweed and seawater were collected and tested for enterococci and faecal coliforms. Some key findings from this work were:

- Enterococci were detected in all sand samples in the range 1-74 CFU/g wet weight.
- Enterococci concentrations in seaweed were highest in older (decayed) samples, reaching $5.8x10^3$ CFU/g wet weight.
- In most cases the concentration of faecal coliforms in a seaweed or sand sample was lower than that of the enterococci, but across the sample types faecal coliform concentrations were higher in decayed seaweed.

During 1997/98, another three beaches were surveyed every three months for one year, these being Long Bay, Mairangi Bay and Rothesay Bay. These three beaches were selected because they had a freshwater stream input, accumulated seaweed and had sufficiently high enterococci concentrations to enable meaningful results to be generated. Samples of seawater, decaying seaweed and the upper layer of sand directly beneath the decaying seaweed, and sand samples from areas free of seaweed, were all collected from several points along each beach and tested for enterococci. Enterococci concentrations in seaweed and sand samples were typically in the range 10^3-10^4 CFU/g dry weight although some higher concentrations were reported. Some overall findings were:

- There was a significant and positive association between enterococci concentrations in the seaweed and sand. This is not surprising given many of the sand samples were in contact with the seaweed while in the beach environment. However, sand samples taken from seaweed free areas contained similar concentrations of enterococci as that taken from seaweed covered areas.
- No other significant correlations were observed, e.g. enterococci concentrations in the sand and water were not significantly correlated.
- Surveys of the freshwater streams discharging into these beach environments identified the water and stream sediments as sources of enterococci, with these discharges perhaps causing occasional elevated levels of enterococci in the receiving seawater.

RAPD-PCR analysis and phenotypic screening of enterococci isolates recovered during these surveys did not suggest that enterococci strains had adapted to specific environments. However, the presence of identical genotypes in seaweed and underlying sand indicated that enterococci could possibly replicate in these environments. Laboratory experiments provided further evidence that enterococci could replicate in seaweed (Anderson, 1999).

6.2 MICROBIAL HAZARDS IN OTHER NZ SEDIMENTS

Liquefaction sediment

The 2010-2011 Canterbury earthquakes led to a large amount of liquefaction in Christchurch. The Ministry for Health study on the potential contamination of silts produced by liquefaction showed that where sewage contamination occurred there was potentially a significant risk to human health (ESR, 2012). In this study, an *E. coli* concentration of 100 MPN/g dry weight (DW) silt, and a virus concentration of not detected/g DW silt, were the criteria selected to assess risk to human health. These criteria were based on the New Zealand Guidelines for the Safe Application of Biosolids to Land (NZWWA, 2003) which is used to manage safe application of sewage sludge to land.

A 20 cm profile was sampled (200-350 g) after removing the top 0.5-1 cm of material to reduce soil-surface contamination. Samples were first collected and tested for *E. coli* on 16 or 21 March 2011, i.e. up to one month after the 22 February 2011 earthquake. After five weeks, *E. coli* concentrations had reduced from >200 to <100 MPN/g dry silt. Sample replicates taken during the same sampling event showed contamination varied across an area of silt.

Field trials were undertaken to study the survival of FIB (*E. coli*, *C. perfringens* spores and F-RNA phage) in liquefaction silt mixed with fresh sewage. The mixture was added to a depth of 5 cm (surface samples, 1-5 cm) or to 20 cm (deep samples, 1-20 cm) during May. Sampling of the surface sample quadrants occurred for 28 days, while sampling of the deep quadrants continued for five months. The freshly amended silt was also analysed for *Salmonella* spp., *Campylobacter* spp., enterovirus, rotavirus, adenovirus and HEV. The results showed that:

- The concentration of *E. coli* in the surface and deep samples were similar over 28 days, with less than 1 log reductions. At depth, this indicator persisted at concentrations ≥100 CFU/g DW silt for three months.
- The concentration of F-RNA phage was low in the sewage-silt mixture, <100 PFU/g DW silt, and it fell below the detection limit (1 PFU/g DW silt) in both quadrants by 28 days.
- The concentration of *C. perfringens* spores did not significantly change over 28 days in the surface samples, nor over the 5-month period at depth.
- Salmonellae were initially present but were not detected in surface samples at 7 days and in deep samples at 14 days.
- *Campylobacter* were initially present but were undetectable in both surface and deep samples at 7 days.
- Viruses were measured by qPCR, therefore the viral genetic material may continue to be detected but it is not known if this material comes from infective (viable) viruses. The surface and deep samples had similar concentrations of Enterovirus and Adenovirus for 28 days, with little decrease (<1 log). Both viruses persisted at depth for the full five-month period and decreased by <1 log compared to the starting concentration. Rotavirus concentrations at day 1 were similar to day 28, but concentrations in the deep samples had decreased by the end of the 5-month period. Results for HEV were highly variable but viral particles were still detectable at five months.

As viruses remained detectable for up to 5 months and had little variation with depth they were still assumed to present a health risk due to their low infectious dose. Depth was not a factor in survival of the faecal indicators, at least during the first 28-day period when this comparison was possible. *Salmonella* was able to survive longer at depth.

Estuarine and river sediments

No published reports or data on FIB and/or pathogenic microorganisms in sediments sampled from New Zealand estuaries were located via the search method.[7]

There are some studies of the presence of pathogenic microorganisms and/or FIB in New Zealand river or stream sediments. In a recent report, sediment was collected from five freshwater stream/river sites in the Manawatū River catchment over a period of 11 months and analysed for *E. coli* (Cookson *et al.*, 2022). Sites were chosen for their differing adjacent landuse, e.g. livestock farming or conservation reserve. The majority of sediment samples (33/35, 94%) were positive for *E. coli* with an average of 89.1 MPN/g dry weight. Many of the presumptive *E. coli* isolates from sediment were positive for known virulence genes by whole genome sequencing (WGS). The *E. coli* concentrations in the river/stream sediment samples were greater than those in adjacent soil samples, but generally an order of magnitude lower than the overlying water (noting these are different matrices).

In another study, sediment samples were collected from the Selwyn, Ashley and Rangitata rivers in Canterbury and cultured for *E. coli* (Davis *et al.*, 2021). Samples (2g wet sediment) were collected in spring and autumn at sites above and below intensive dairy operations. The concentrations of *E. coli* in sediment samples varied between the sites and seasons, ranging between 200 and 7100 CFU/ml in samples collected above intensive dairy operations, and in the range 230–273,300 CFU/ml below intensive dairy operations. The sediment samples yielded higher levels of *E. coli* than associated water samples.

[7] Data on estuarine sediments are likely to exist, e.g. in repositories in universities, research institutes, local authorities or government departments. An extensive search of these sources for these data was not undertaken.

In a third study, surficial sediment grab samples were collected in March and April of 2011 and 2013 from three sites along the Avon River, Christchurch during and after discharge of untreated human sewage resulting from the earthquakes (Devane *et al.*, 2019). Samples were analysed for *E. coli*, F-Specific RNA bacteriophage, *Campylobacter* spp., *Giardia* spp. and *Cryptosporidium* spp. *E. coli* was detected in all sediment samples from all sites.

6.3 CLIMATE CHANGE IMPACTS

New Zealand's coastal climates will continue to become warmer. However, it is difficult to predict how this might change the micropsammon population, and the presence of pathogenic microorganisms, since there are many interacting factors, e.g. weather events, infrastructure resilience and human activities.

Two reviews specifically discussed the impact of increased temperatures, altered precipitation patterns, rising sea levels and changed wave activity in the context of sand safety (Brandão *et al.*, 2022; Weiskerger *et al.*, 2019). Some general points emerge:

- Increased temperatures will not necessarily increase growth rates of bacterial pathogens, although it will make conditions more favourable for some such as allergenic fungi and *S. aureus*. Temperature is only one environmental variable that determines survival and growth. Some environmental microorganisms prefer cooler temperatures. However, consistently warmer temperatures will extend the range of microbes currently more abundant in tropical and subtropical zones (e.g. *Vibrio* spp.).
- Increased solar irradiation is unfavourable for microbial survival on the sand surface.
- In areas experiencing increased precipitation and/or frequency of severe weather events, the entry and survival of pathogenic microorganisms (and supporting nutrients) into the beach environment is likely to increase. This will particularly affect recreational beaches where there is risk from infrastructure failure or sewage/stormwater overflows. Storms often cast seaweed onto the beach, which protects sand microorganisms and supports bacterial growth. However, very severe weather events can also lead to significant beach erosion, which can remove contaminated sand (Roca *et al.*, 2019).
- Drought conditions might concentrate microorganisms in beach inflows.
- Increased sea levels and wave energy will also impact sand microbial communities, chiefly through shifting the sand-water continuum landwards. Changes that result in less wave energy will allow microorganisms to accumulate in sediments and form biofilms, which prolong survival.

While not specifically considering beach sand, another review discusses the role of climate change in the emergence and global spread of fungal pathogens (Nnadi and Carter, 2021). Examples included were species of *Candida* and *Cryptococcus*, both of which were identified as potentially important sand pathogens (Section 5.4). The reviewers note that emerging pathogenic fungi pose a significant threat to human health because they commonly live in the environment (and do not require host-to-host transmission to establish an infection) and produce large quantities of infectious spores. Plus, there are no vaccines and limited antifungal treatments.

Exposure to beach sand will also change through alterations in human behaviour. People are likely to visit recreational beaches for longer periods during the year as warm periods are extended. However, the length and timing of these visits might change, e.g. as people seek to avoid very hot weather.

7. DISCUSSION

Summary

Microbiological pathogens in sand do present a potential health risk, noting there is limited epidemiological evidence and important data gaps that hinder QMRA. Enterococci are the recommended indicators for the potential presence of faecal-associated pathogens in marine beach sand. *E. coli* might also be considered as an indicator of faecal contamination in freshwater beach sands. These FIB indicate elevated risk from fresh faecal contamination. Complementary tools, including sanitary surveys and molecular methods like MST, provide a more complete body of evidence to assess risk.

The health risks from microbiological pathogens naturally present in beach sand, or introduced with beach visitors, are uncertain. The presence and concentration of these microbes in sand cannot yet be linked to infection risk.

Further information is required on the presence and survival of FIB and pathogenic microorganisms in sand within a New Zealand context. Research can be directed towards filling data gaps that currently prevent QMRA.

There are a range of microbiological hazards that could be present in beach sand. These might be considered as two groups, (1) those associated with faecal contamination, and (2) those naturally present or introduced through human activities on the beach.

7.1 MICROBIOLOGICAL HAZARDS FROM FAECAL CONTAMINATION OF SAND

The presence of faecal contamination in sand presents the greatest risk for adverse health effects through sand contact, particularly if the contamination is from sewage. However, there is currently only limited epidemiological evidence linking adverse human health outcomes with faecal contamination in sand, as indicated by elevated sand concentrations of enterococci. The main reason is the difficulty in separating exposure to sand from water since people typically make contact with both environments while visiting beaches. Outbreaks of salmonellosis have been caused through exposure to playground sand contaminated by wildlife faeces.

There is sufficient evidence from epidemiological studies of recreational water exposure to support the use of FIB to indicate elevated risk from faecal-contaminated sand. However, FIB may not indicate the potential for faecal-associated viruses or protozoa to be present from aged faecal contamination. In addition, most of the epidemiology studies used to underpin the enterococci guidelines for recreational water quality are based on human faecal contamination (WHO, 2021), yet contamination from animal faeces must also be considered in New Zealand.

Some test methods can help address the above issues. The liquefaction sediment study showed that *C. perfringens* spores could be suitable to indicate aged faecal contamination from sewage (ESR, 2012). However, *C. perfringens* is both environmentally ubiquitous and can be an unreliable marker for herbivorous animal faeces (Vierheilig *et al.*, 2013). Some MST markers are useful indicators of aged human faecal contamination. More widely, MST is a useful tool to identify where faecal contamination might be entering a beach sand environment since sanitary surveys may not identify all relevant sources of contamination (Leonard *et al.*, 2021).

The above also applies to assessing the safety of sand being considered for relocation to a recreational beach, i.e. identifying sources of contamination via sanitary surveys, using FIB

to indicate faecal contamination, and applying tools like MST to provide more complete information about health risks.

The WHO recommend using enterococci as a faecal indicator. Enterococci are used for marine waters because they are more tolerant of saline conditions. *E. coli* was shown to be a useful indicator for pathogen survival in New Zealand liquefaction silt and freshwater (ESR, 2012; McBride *et al.*, 2002). There are freshwater sandy beaches in New Zealand that are used for recreation. Thus, *E. coli* might also be evaluated as an indicator for faecal contamination of sand from these environments, and potentially coastal beach environments with low salinity (e.g. upper estuarine/harbour areas). One further point raised through this review is the potential for seaweed to harbour high concentrations of enterococci by providing suitable conditions for bacterial multiplication. These bacteria can be spread into the wider beach environment where they might indicate elevated health risk. A combination of visual inspection and molecular methods (e.g. MST and NGS) can provide more information towards understanding whether there are health risks.

The WHO (2021) guideline value for enterococci assumes that the ratio of pathogen:enterococci in sand is the same as in water. The decay rates of FIB and faecal-associated pathogens tend to be slower in sand compared to water, particularly since bacteria can form longer-lasting biofilms in sand. Investigations of microbiological decay rates using New Zealand sands under New Zealand climatic conditions would improve decision-making. The panel of faecal-associated microorganisms selected for such studies could be guided by other New Zealand studies, such as the recent surveys of New Zealand freshwaters (Leonard *et al.*, 2020b; Leonard *et al.*, 2021).

Planned sampling of high-risk beach sand areas may be a useful health protection strategy. The microbiological quality of sand could be monitored where contamination is likely, e.g. where there are known sewage and stormwater overflows or after a contamination event. As shown in Section 3.1.2, sand can retain microorganisms while contamination in the water column dissipates. Thus, sand sampling can assist with understanding any ongoing risks from a contamination event after the water quality criteria may have returned to acceptable levels.

7.2 OTHER MICROBIOLOGICAL HAZARDS

The health risks from sand containing any members of the second group of microbiological hazards, those naturally present or introduced with beach visitors, are uncertain. The adverse health effects range widely, encompassing both serious and comparatively mild conditions. Host susceptibility and strain variation impact the probability of an infection, the probability of that infection proceeding to illness, and the severity of illness. The dose response is not well characterised for some in this group (notably fungal pathogens). Transmission pathways include sand ingestion, sand inhalation and skin contact. Work undertaken in other countries suggest that fungi (total, or some specific groups) and *S. aureus* could be useful indicators of sand microbiological quality. However, the presence and concentration of these microbes in sand cannot yet be linked to infection risk, which makes it difficult to set microbiological guideline values.

While evidence of a correlation between sand fungi concentrations and human infection is lacking, Portugal has adopted guidance values for total fungi in sand as one of their sand safety indicators (WHO, 2021). Under this programme, 80% of samples must contain a total fungal count of ≤490 CFU/g, and a mean guidance value of 89 CFU/g is used as an indicator of beach sand safety. Earlier work in Portugal suggested threshold values for specific fungal groups, proposing that 95% of samples should contain ≤15 CFU/g yeasts, ≤17 CFU/g potential pathogenic fungal species (e.g. *Aspergillus*, *Fusarium*) and ≤8 CFU/g dermatophytes (Sabino *et al.*, 2011).

7.3 MONITORING SAND QUALITY

At a 2014 international meeting, experts in recreational beach water quality and environmental mycology agreed on some general recommendations for beach sand sampling programmes (Solo-Gabriele *et al.*, 2016). In summary, it was recommended that:

- Microbial monitoring in sand be included as part of routine recreational beach health assessments, for both marine and freshwater beaches.
- Microbial monitoring be informed firstly by a sanitary survey of the beach environment to identify anthropogenic and natural contamination sources (e.g. groundwater, stormwater, faecal inputs), and any mitigation measures in place (e.g. rubbish bins, showers, wastewater infrastructure).
- The location of sample sites is informed by the sanitary survey and where visitors congregate, ensuring hotspots of contamination are included.
- The sample collection method should consider both shallow sand and deep sand, and make use of pooled samples from multiple sites where there are no obvious sources of contamination.
- The microbial targets be selected through considering the cost of testing, the speed of receiving results and the connection with human health. Testing for FIB might be complemented with tests targeting specific pathogens. Selection of the latter can be informed by the sanitary survey and historical information (contamination sources, outbreaks) and might consider protozoan parasites, viruses or fungi.
- A tiered approach be adopted, firstly testing for concentrations of FIB and total culturable fungi, then investigations using source tracking methods (e.g. microbial source tracking, fungal identification). The third tier involves testing for specific aetiological agents of disease.

The same group identified that, as wastewater infrastructure and treatment continues to improve, non-point contamination sources are becoming relatively more important for beach sand safety compared to point sources. Pathogenic microorganisms can be present in beach sand in the absence of sewage contamination. They suggested, on this basis, that the use of FIB (enterococci, *E. coli*) has limited value and it was important to undertake research to determine which other microorganisms could serve as useful markers of sand hygiene. However, there were several data gaps that prevented this, some of these being:

- Studies of alternative faecal indicators are needed to demonstrate that these (a) are consistently and exclusively associated with a source of human pathogens, (b) are unable to multiply under the environmental (beach) conditions, and (c) survive similarly in the source and under subsequent environmental conditions to the human pathogens.
- Evidence of links between specific aetiological agents of disease, their presence and survival in sand, and human infections/illness are needed. This requires epidemiological and environmental studies, further considering differences between freshwater and seawater beaches.
- Dose response data to inform QMRA are needed. These also need to consider the exposure route (e.g. dermal exposure including open wounds, ingestion).

Further to this, it has been recommended that QMRA models incorporate emerging and changing conditions as a result of climate change (Brandão *et al.*, 2022).

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APPENDIX A: SCOPE AND METHOD

A.1 Project scope

Table 1 clarifies the scope of this review as applied at the outset of the project. The review initially focused on attributes 'in' scope but as the work progressed, some literature considering attributes out of scope were included, e.g. to provide information where there were data gaps.

Table 1. Scope of this review

ATTRIBUTE	SCOPE	REASON
Types of beaches		
Freshwater and marine sands	In	Estuaries, harbours, and freshwater rivers and lakes can also have sandy beaches and become contaminated.
Pebble beaches	Out	High surface area of pebbles decreases survival from sunlight, and desiccation. Less attractive for playing and contact.
Muddy estuaries, liquefaction solids, mud flats	Out	High organic content affects survival environments. Complex oxidation/reduction chemistry during tidal processes.
Types of microbiological organisms		
Faecal indicator micro-organisms: Enterococci, faecal coliforms, *E. coli*	In	There is a known relationship between concentrations of *E. coli*, enterococci and some microbial pathogens in raw sewage, but survival in sand will differ from water.
Pathogenic bacteria, fungi, viruses, protozoan parasites likely to be present in NZ	In	Only those that are likely to be present in NZ.
Phytoplankton causing harmful algal blooms	Out	Harmful algal blooms are already monitored and managed. The hazard is the toxin (chemical hazard).
Microorganisms primarily causing diseases in people who have recently travelled overseas	Out	These are non-endemic diseases for NZ, e.g. typhoid, cyclosporiasis. It is acknowledged that these pathogens could be present in sewage as a result of infected cases being in NZ, and so might temporarily enter coastal environments.
Microorganisms causing tropical diseases	Out	See above.
Non-pathogenic microorganisms	Out	No risk to human health.
Sources of microbial pathogens		
Point source faecal contamination	In	Sporadic events or (semi)continuous e.g., broken sewer infrastructure, combined sewer/stormwater flows, sewage discharges, processing discharges. Also point-source contamination from concentrations of animals e.g., bird nesting grounds, stormwater from animal holding or processing sites.

ATTRIBUTE	SCOPE	REASON
Non-point source faecal contamination e.g. domestic and feral animals or runoff from agricultural land	In	Runoff from nearby land-based activities e.g., parking lots, agricultural land. Upwelling from groundwater into the beach sand will also be considered.
Naturally occurring microorganisms	In	Natural sand inhabitants and those that might persist in the sand after being introduced from the aquatic environment.
Resuspension of sediment by wave action or swimmers	Out	Ingestion from swallowing water, although water quality parameters are sampled in the absence of sediment suspension.
Resuspension of sediment from tidal action in estuaries	Out	Important as estuaries are a common place for children to play as less wave action, often shallow and warmer but estuarine chemistry is complex with changes in oxidation state of the sediment potentially contributing to desorption and absorption of pathogens.
Literature subject matter		
Literature reviews	In	Effective method of collating evidence.
Studies of sand in temperate climates*	In	Relevant to NZ climate.
Studies of sand in tropical or extremely cold climates*	Out	Not relevant to NZ at present, but overview of tropical climates due to climate change will be provided.
Evidence of gastroenteric illness or other adverse reactions (e.g., skin irritation)	In	Evidence that microbial contamination of sand is a potential health risk.
Modelling of human health risk (e.g., QMRA)	In	Recommended as an approach by the WHO.
Sandpits	Out	Unknown provenance. Contamination is more likely to occur *in situ* (e.g. cats or birds defecating in sandpits).
Imported sand	Out	Unknown provenance.
Survival of microorganisms in sand compared to water	In	Some micro-organisms are more likely to survive longer in the presence of sand compared to the water column.
Prevalence/concentration of microorganisms in sand in NZ	In	Shows whether microorganisms have been detected in sand. However, only reports will be reviewed to ensure there is context. Isolated data often lack suitable context (e.g., source, risk).
Prevalence/concentration of microorganisms in sand overseas	Out	See above. These data will be considered if there are no prevalence data available for NZ and more suitable data are not available (e.g., where there is evidence that the presence of these microorganisms presents a risk to human health).

* As guided by Zone C Köppen-Geiger climate classifications (Beck *et al.*, 2018), see also http://www.gloh2o.org/koppen/.

A.2 Review method

A reference library was established from the references cited in Chapter 7 of WHO (2021). This library was expanded, as described below.

Web of Science[8] and PubMed[9] are two citation search engines that together index scientific publications spanning the environment and human health. These libraries were searched during August and September 2022, using the following key word sets:

Hazard	escherichia OR enterococci OR enterococcus OR coliform OR bacteria OR parasite OR virus OR pathogen OR protozoa OR fungi OR amoeba
Health	outbreak OR infection OR rash OR enteritis OR diarrhoea OR diarrhea OR illness OR fever OR allergy OR allergen OR respiratory OR vomit
Exposure 1	sand OR beach
Exposure 2	“New Zealand” AND (sediment OR sand) AND (harbour OR estuary OR estuarine)

These keyword sets were applied in the following combinations:

- Hazard + Exposure 1
- Health + Exposure 1
- Hazard + Exposure 2
- Health + Exposure 2

When the above searches retrieved thousands of results, the first 50 were scanned and refinements to the search strings were made. Tools available in Web of Science were used to create subsets for further searching (e.g. review articles). Potentially relevant citations were selected based on the title. After removal of duplicates, each citation was assessed for relevance based on the abstract. Additional articles were identified using reference lists and PubMed’s ‘cited by’ function.

Additional references were located through the reference lists of retrieved articles. As part of quality control, if a review cited results from a study of interest, the primary paper was usually retrieved to ensure the data were described correctly.

Additional searches of the scientific literature were conducted as needed e.g., for further information on specific pathogens or events.

[8] https://www.webofscience.com published by Clarivate Analytics.
[9] https://pubmed.ncbi.nlm.nih.gov/ published by the National Center for Biotechnology Information, US National Library of Medicine.

APPENDIX B: SELECTION OF MICROBIAL HAZARDS FOR PROFILING

As a first step towards determining the microbial pathogens to be included in this review, data on notifiable GI diseases and case-reported recreational water exposure were considered (Table 2).

Table 2. Notifiable GI diseases in New Zealand for 2019: Reported cases and recreational exposure to water as a risk factor

CAUSATIVE ORGANISM	NOTIFIED CASES	RATE PER 100,000	PERCENTAGE REPORTING RECREATIONAL WATER CONTACT[1]	PERCENTAGE OF CASES WHO ANSWERED THIS QUESTION[2]
Giardiasis	1749	35.6	38.4	48.0
Cryptosporidiosis	1035	21.0	28.1	47.5
Pathogenic *E. coli* infection (STEC)	1101	22.4	26.3	69.7
Salmonellosis	1188	24.2	23.9	66.1
Campylobacteriosis	6202	126.1	23.3	34.4
Yersiniosis	1186	24.1	21.7	46.3
Shigellosis	222	4.5	19.1	59.0

Source: ESR Epidemiology Team, 2021

1 The percentage of cases answering 'yes' based on the total number of cases that provided information on recreational water contact.

2 The percentage of cases that provided information on recreational water contact.

Other considerations were:

- Geographical: There are a number of diseases that are not considered to be relevant in New Zealand but might be elsewhere. Examples include vectorborne pathogens such as *Leishmania* (causes leishmaniasis) and *Bartonella bacilliformis* (causes carrion's disease or oroya fever), which are problematic in some coastal regions of the world. Data from tropical beaches are also not relevant for New Zealand.
- Non-endemic diseases: It is difficult to determine whether there could be risks in New Zealand from returning travellers infected with pathogens not endemic in New Zealand. Examples include toxigenic *Vibrio cholerae* (causing cholera) and typhoidal salmonellae. At this point, such pathogens have not been included in this section since the risk appears higher from endemic microbial pathogens.

The following microbiological pathogens have been excluded although there remains potential for any of these to cause disease among beach visitors.

***Shigella* spp.** have been excluded because the majority of New Zealand cases are caused by exposures in other countries. For example, in 2019 there were 222 reported cases of shigellosis, 193 (86.9%) of these reported whether they had travelled overseas during the incubation period for the disease, and 117/193 (60.6%) had (ESR Epidemiology Team, 2021). Ten further cases had a prior history of travel. While currently excluded, the potential for a shigellosis outbreak to occur in a beach environment through environmental contamination cannot be completely ignored, given the statistics above. Humans are the primary reservoir for *Shigella* spp., with human faeces being a source of environmental contamination. Shigellosis (also called bacillary dysentery) occurs through faecal-oral transmission, usually after consumption of contaminated water or food (e.g. from an infected food handler).

Very similar to *Shigella* spp., infections from **non-typhoidal salmonellae** (*Salmonella* Typhi and *Salmonella* Paratyphi) were excluded because most New Zealand cases are caused by exposures in other countries. In 2019 there were 18 paratyphoid cases of which 14 had travelled overseas, plus 55 typhoid cases of which 42 reported overseas travel (ESR Epidemiology Team, 2021). Human faeces is the source of environmental contamination.

***Clostridium* spp.** are sometimes used as indicators for faecal contamination (Heaney *et al.*, 2012; Shah *et al.*, 2011). They are spore-formers, found in the gastrointestinal tract of animals and are environmentally stable, so might be considered to be conservative indicators for longer-surviving pathogenic microorganisms. However, they might not be a useful indicator for ruminant faecal contamination (Vierheilig *et al.*, 2013). Only some clostridia are pathogenic (Guo *et al.*, 2020).

***Legionella* spp.** are found in freshwater although human illness has been associated with handling garden potting soil containing one species, *Legionella longbeachae* (Fields *et al.*, 2002). With a preference for warmer water temperatures, legionellosis cases have also caused through contact with water distribution systems (Burillo *et al.*, 2017). Cases in New Zealand are report exposure to compost, potting mix or soil, or to water from hot water systems, spas/pools, air conditioning, etc. (ESR Epidemiology Team, 2021).

Listeria monocytogenes is environmentally widespread but listeriosis is a foodborne disease (Buchanan *et al.*, 2017).

***Leptospira* spp.** are zoonotic pathogens with human cases usually associated with contact with animals or flooding events (Samrot *et al.*, 2021). Only some species have been associated with human disease, most commonly *Leptospira interrogans* and *Leptospira borgpetersenii* (ESR Epidemiology Team, 2021).

Hepatitis viruses other than HAV. Of the hepatitis viruses, exposure to HAV or HEV in human faeces is a cause of disease and in New Zealand, HAV infection is more important (Castaneda *et al.*, 2021; ESR Epidemiology Team, 2021). Some types of HEV can also be shed by infected animals (primarily swine) but, with many human cases being asymptomatic, the epidemiology and burden of disease in New Zealand is not well understood (King *et al.*, 2018).

Faecal-oral transmission is important for infection by **rotavirus**, and this is a globally-important cause of diarrhoea (Omatola and Olaniran, 2022). However, other viruses were selected for coverage in Section 5.2 of this report. A rotavirus vaccine was introduced into the New Zealand child immunisation during 2014.

Respiratory viruses circulating in New Zealand such as SARS-CoV-2 and respiratory syncytial virus (RSV). Person-to-person transmission is the most important transmission route and while faecal shedding can occur, the shed viruses might not be viable. There is potential for these viruses to be transferred to sand with respiratory droplets, where they might survive or be inactivated by heat and UV (Efstratiou and Tzoraki, 2021). However, such viruses will not be a priority for sand surveys.

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