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Present status of effect of microorganisms from sand beach on public health

Emmanuel Velonakis[1,2], Dimitra Dimitriadi[1*], Emmanuel Papadogiannakis[3], Alkiviades Vatopoulos[1,2]

[1]*Department of Microbiology, National School of Public Health, Athens, Greece*

[2]*Central Public Health Laboratory, Vari-Attica, Greece*

[3]*Department of Veterinary Public Health, National School of Public Health, Athens, Greece*

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ABSTRACT

Microorganisms are significant components of beach sand. According to the research, all kind of microorganisms have been isolated from beach sand; certain genera and species are potential pathogens for humans and animals. In resort areas, especially during the summer, certain infections (*e.g.* gastroenteritis and dermatitis) are usually related to polluted bathing water. Lately, the interest of scientists is also focused on the potential association of some of the above diseases with the beach sand. Relatively, recent epidemiological studies in the USA revealed positive correlation between time spent at the beach and gastroenteritis. New parameters such as wind blowing and beach users' density are also introduced for discussion in association with the sand microbial load. Regarding the preventative measures, the microbiological quality of beach sand can be improved by raising the general level of hygiene, as well as by using simple methods, such as sweeping and aeration of the sand, together with constant beach supervision.

1. Introduction

According to the prevailing scientific opinion, beaches comprise the unconsolidated sediment that lies at the junction point between water (sea, lake, river) and land. The sediment is usually composed of sand, mud and/or pebbles.

Beaches are significant for recreational activities, especially in northern countries where time spent on the beaches is usually more than the one spent in the water because of low water temperatures. In recent years, special attention is being paid to the care and cleaning of the coasts, since many stakeholders have turned their interest to risks from environmental exposure due to the fact that tourism is a very powerful sector of the economy for many countries.

*Corresponding author: Dimitra Dimitriadi, Department of Microbiology, National School of Public Health 196, Alexandras Ave., 115 21-Athens, Greece.
E-mail: dimdimitriadi@hotmail.com

Recently, Centres for Disease Control reported that the incidence of infections associated with recreational water have steadily increased over the past several decades, as a result of emerging pathogens, increases in aquatic activities and better disease reporting[1].

According to the international bibliography, many microorganisms have been isolated from the sand as well. A number of species and genera of these microorganisms are potential pathogens and feasibly can come into contact with humans through sand (Tables 1 and 2).

Contamination sources can be either point or non-point ones. Point sources are located in a specific position and are easily identifiable. In contrast, non-point sources are disseminated and hardly recognizable. The microbial load of non-point sources (*e.g.* runoff from urban and rural areas, leaks from biological cleaning systems and drainage systems, discharges from boats and atmospheric deposition of aerosols) is large[2]. Epidemiological studies on beaches with non-point source pollution are fewer and have dubious success in correlating faecal indicator

Table 1
Bacteria isolated from beach sand.

Microorganisms	Origin	Main points	References
Escherichia coli	Microorganism of human and animal enteric flora	Humidity, temperature, UV rays and concentration of dissolved organic carbon affect viability of microorganism	[17,18]
Enterococcus spp.	Microorganism of human and animal enteric flora	*Enterococcus* spp. drug resistant strains are often isolated from beach sand	[32]
Clostridium spp.	Microorganism of human and animal enteric flora	Could be a reliable indicator of faecal contamination of sand	[23]
Staphylococcus spp.	Microorganism of the human and animal normal flora	They dominate in sand	[23]
Pseudomonas aeruginosa	Enviromental microorganism (water, soil, plants)	In a study along the coasts of Gaza Strip, it was isolated from all samples of sand and water	[37]
Enteric bacteria *Salmonella* spp., *Campylobacter* spp., *Shigella* spp.	They are discharged from human and animal faeces, except *Shigella* which is discharged only from human faeces	Despite the fact that have been isolated from sand samples, it is maintained that they do not pose an obvious danger for beach users	[23]
Vibrio spp.	Microorganism of the water (brakish, sweet)	Different species of *Vibrio* spp. have been isolated from water and sand	[23,37]

Table 2
Viruses, parasites and fungi isolated from beach.

MIcroorganisms	Origin	Main points	References
Viruses: enterovirus	Microorganisms which are discharged from human and animal gastrointestinal tract	Have been isolated on the shores of the Black Sea	[51]
Parasites: *Toxocara* spp., *Ankylostoma* spp., *Nocardioides*	Microorganisms which are discharged from human and animal gastrointestinal tract	Several parasites have been isolated from beach sand	[41,23,43]
Fungi: *Trichosporon* spp., *Candida* spp., *Trichophyton* spp., *Microsporum* spp., *Epidermophyton* spp., *Rhodotorula* spp., *Penicillium* spp., *Aspergillus* spp.	Fungi are classified as anthrophilic, zoophilic and geophilic	Fungi are often found in the sand and survive longer as compared to other microorganisms	[23,44,46]

bacteria (FIB) abundance to bather health outcomes of enteric illness, respiratory and skin infections. Howsoever, FIB absence do not always exclude the presence of other pathogen microorganisms in sea water samples examined for their microbiological quality. Nevertheless, at a beach in California, affected primarily by non-point source pollution, although no association was found between the abundance of traditional FIB and bather's health problems, there has been an increased incidence of diarrhoea and skin rashes when compared to non bathing beachgoers[1].

Infections to beach users, such as gastroenteritis and viral infections, especially during the summer months, in recreational beaches are normally associated with water pollution. However, in recent years the interest has also been focused on the relationship of these diseases with sand.

Thus, lately, epidemiological investigations on beaches in the USA found a positive correlation between time spent on the beach and the incidence of gastroenteritis[3,4]. Despite this, more studies are needed in order to prove or reject this possibility[4].

Currently, no U.S. federal guideline is available for assessing risk of illness from sand at recreational sites. An National Institute of Environmental Health Sciences funded researcher recently published reference levels for the risk of human illness from pathogens in beach sand. The researchers used quantitative microbial risk assessment and Monte Carlo simulations to calculate beach sand pathogen levels corresponding to the 1.9% EPA reference risk for marine water, which is equivalent to 19 illnesses per 1 000 swimmers. For beach sand, they calculated reference levels of about 10 oocysts per gram of cryptosporidium, about 5 most probable number per gram for enterovirus, and less than 1 000 000 colony forming units per gram for *Staphyloccocus aureus (S. aureus)*[5].

This review has been written in order to bring to light the results of the research carried out to date for the microorganisms isolated from sand, the conditions that favour their survival and the interventions to be undertaken in order to maintain and improve the microbiological quality of the sand.

2. Classification and significance of soil

There are several classification systems concerning soils. According to the United States Department of Agriculture Soil Taxonomy developed by United States Department of Agriculture and the National Cooperative Soil Survey, soils of interest are classified as below: 1-2 mm: very coarse sand; 0.5-1 mm: coarse sand; 0.25-0.5mm: medium sand; 100-250 μm: fine sand; 50-100 μm: very fine sand; 2-50 μm: silt; <2 μm: clay.

The terms sand, silt, and clay refer to relative sizes of the soil particles. Sand, being the larger size of particles, feels gritty. Silt, being moderate in size, has a smooth or floury texture. Clay, being the smaller size of particles, feels sticky[6]. Silt and clay are easily transported in water

and are fine enough to be carried long distances by air as "dust" and due to their small size it should be considered the possibility that those with an aerodynamic diameter of <5 μm could not be excluded from reaching the lower respiratory tract.

The soil habitat represents a unique but extensive environment in which microorganisms live and carry out biochemical reactions, critical to the maintenance of ecosystems. Attachment of microorganisms to soil particles represents an advantage in some ways. They may obtain some essential element from the particle grains, they might obtain energy from organic or inorganic molecules tightly sorbed to the mineral grain surface or they might benefit from living in a chemically richer environment due to enhanced concentrations of soluble nutrients in the proximity of the grain surface. In some cases, attachment to particles might provide partial or complete protection from grazing by bacterivorous organisms[7].

A number of factors influence the attachment and permanent of bacteria with soil particles. In addition to particle composition, particle size seems to play an important role in determining the distribution of microbial population in soil aggregates. A number of studies have shown that both the cell number and the bacterial biomass are tend to be most concentrated in the smaller size silt and clay fractions. Analysis of the distribution of microbial enzyme activities suggest that the bacterial activities are dominant in the silt and clay fractions, whereas enzyme activities that indicate fungi are highest in the sand. There is a good reason why clay fractions would have the maximum interactions with bacteria. The particles small size yields an enormous surface area per unit weight of solid, and the crystal structure of clays tends to engender a strong net negative charge on the surface that can attract nutrients, organics, and under the right circumstances, the bacterial cell themselves. The influence of the finer texture material is a combination of a surface area increase and the specific mineralogy of the particles (which is not discussed in this article)[7]. Nevertheless, a study of the association between the size of the sand particles only and the survival of the microorganisms, showed that the greatest survival was of the bacteria attached to particles 850 μm-2 mm in size[8].

3. Methods

This review draws from peer-reviewed publications indentified from Science Direct and Pubmed research databases. References here included were mainly dated after 2000. Earlier publications were also included, when identified to be important. Search strategies included key words and other relevant words with various combinations. The PubMed "related articles" function was used to search for other relevant articles not retrieved in initial keywords searches. This review included original studies and review articles.

4. Isolation and detection of microorganisms

FIB density is widely used to make microbiological water quality decisions at beaches, but it remains unclear whether their presence in sand is indicative of increased human health risk. One complicating factor, in understanding their significance, is the absence of a widely accepted method for FIB extraction from the sand. So, examination methods published range from simply shaking the sample by hand, to carrying out complex protocols involving use of sonication, mechanical shakers and sophisticated buffers. Methods based on shaking are most frequently used, but even they vary in duration/type of shaking, type and volume of eluant, mass of sand used, number of rinse steps and using of pre-filtration. One study has compared twenty-two methods of extracting enterococci and *Escherichia coli (E. coli)* from sand. The simplest extraction method with the highest recovery rates, consisted of two minutes of hand shaking with one rinse step, a 30 seconds settling time, and a 10:1 eluant volume to sand weight ratio[9]. However, it is of grate importance, the characteristics of the sediments, whether they are consisted mainly of sand or contain considerable proportions of silt and clay, in determining the most efficient method for the separation of micro-organisms from coastal sediments[10]. As studies begin to follow the same method, data from different studies will become comparable. *Enterococcus faecalis*, like other non-sporulating bacteria, can respond to environmental stressors by altering its physiology to a distinctly different viable but nonculturable (VBNC) state, whereby cells are vegetative and not culturable. Likewise, in marine waters, enterotoxigenic *E. coli* strains have been documented entering the VBNC state upon exposure to sunlight and subsequently persisting in the environment while retaining toxicity. These studies highlight one of the flaws of the culture-based method of indicator bacteria detection[1]. The same VBNC state has been observed in *Salmonella*[11], *Campylobacter jejuni (C. jejuni)*[12], *Vibrio cholerae*[13] and *Vibrio vulnificus*[14].

Molecular technologies are also a useful tool for detection of pathogens from environmental samples. Polymerase chain reaction is the most widely used method. This technology is allowing researchers to rapidly and specifically target microbes of public health concern, including those that were previously unexamined because

of the inability to culture them. One major drawback is that DNA detection of the pathogenic microorganisms is not necessarily indicative of their viability or pathogenicity. Despite all the improvements in the technical isolation of pathogenic microorganisms, the collection and processing of microorganisms require continual modification. The soil and sediment are generally characterised by heterogeneity and their chemical and physical composition varies significantly, thus causing difficulties in creating a specific protocol for the collection and processing of the samples[2].

5. Pathogenicity of microorganisms

It is not enough for a methodology to detect the presence of an infectious bacterium, since there is a great diversity of strains which are not equally pathogenic. For instance, *Vibrio parahaemolyticus* (*V. parahaemolyticus*) is often found in marine environments, but few genotypic distinct strains are responsible for infecting humans. For this reason, rapid detection tools should dispose the necessary sensitivity to detect low numbers of a pathogen, as well as the competence to detect the presence of specific strains of pathogens which can express virulence genes capable of human infection[2].

6. Microbial markers for sand

FIB and other pathogens have been isolated from the sand of different kind of coastlines. However, the possibility of some of such microorganisms to cause infections to users of the beach remains unproven and the real extent of their threat to public health remains unknown.

In addition, as shown in some studies, the extended survival of some of these microorganisms in the sand, such as *E. coli*, enterococci and fungi, independently of the presence of a polluting source, as well as the capability of their cells to proliferate dispute their use as FIB[15]. New microbial indicators might be adopted in the future for better identification and monitoring contamination of the sand. Amongst other features, these indicators should not be able to multiply in the natural environment; they should be found in small concentrations in intact environments and in high concentrations in waste water. Proposed microbial indicators are bifidobacteria, *Clostridium perfringens (C. perfringens)*, F RNA and coliphages[2].

A recent study searching for alternative indicators of disease-risk from non-enteric pathogens at the beach revealed high numbers of *S. aureus* and *Pseudomonas aeruginosa (P. aeruginosa)*. Factors affecting the survival of those bacteria were investigated, simultaneously with a potential faecal indicator, *C. perfringens*. Results indicated greater survival and proliferation of *S. aureus* and *P. aeruginosa* in sterile beach sand than seawater. *C. perfringens* remained consistent with initial numbers. The colonization of sterile wet and dry sand by these bacteria, demonstrated the ability of sand to filter bacteria out of the water. Inversely, waves, tidal cycles and runoff, washed bacteria out of the sand in the shoreline water. The findings of the study support that the use of these potential pathogens in periodic sanitary evaluation of beach sand quality, in the absence of ideal indicators of non-enteric, is indicated[8].

7. Factors influencing the distribution and survival of the microorganisms

The growth of microorganisms in the sand is limited by insufficient nutrients and competition with the native microbial flora. However, enterococci typically display tolerance to extremes in pH, temperature, salts, and detergents and their surface hydrophobicity makes them more successful at utilizing starvation and biofilm modes of growth[1,16].

Studies have shown that there are a number of factors which affect the concentration of FIB in the sand. Thus, humidity, temperature, UV rays or a concentration of dissolved organic carbon are some of the factors affecting the viability of microorganisms[17,18].

Certain researchers argue that there is a difference in the decline rate of microorganisms-indicators at 20 ℃ in relation to 10 ℃, with faster decrease of their concentration at 20 ℃, while others do not find such correlation[17]. The survival of pathogens *S. aureus* and *P. aeruginosa* has also been investigated at 20 ℃, 30 ℃ and 40 ℃ in the wet sand. *S. aureus* increased 68-fold within 5 d at 30 ℃. At 20 ℃, the survival in the sand was four time less than that at 30 ℃. Survival in the sand at 40 ℃ was insignificant when compared to those at 20 ℃ and 30 ℃. For *C. perfigens*, the values remained fairly consistent with the initial values, respectively across all temperatures[8]. Concerning the effect of the temperature, according to research carried out by Ishii *et al.*[19], the frequency of *E. coli* isolation was higher from July to September due to more favorable temperatures. On the contrary, another research showed that colony forming units of *E. coli* and enterococci decreased along the summer season (June to August). This fact could be explained by the decrease of surface runoff and the increase of solar duration and intensity along the sampling periods[20].

Regarding humidity, again, there are contradictory findings, as there are surveys which show higher

concentrations of microorganisms in the sand that was rinsed by the seawater due to the positive effect of humidity on growth, while in others, the number of microorganisms in dry sand is higher in relation to wet one. This must be due to more intensive human activity in dry sand and runoff from adjacent areas. Moreover, comparative observations of micro-grazers in wet and dry sand showed that dry sand contained fewer and smaller protists compared to wet one[3,20-22].

UV rays cause inactivation of microorganisms, with *E. coli* being more resistant than enterococci. However, it seems that the sand has a protective role for microorganisms[8,18].

Tidal phenomena is another factor proposed as facilitating the dispersion and survival of microorganisms[23]. Sediment samples were analysed in one study in the United Kingdom for thermophilic campylobacters and FIB under various tidal conditions over a 12-month period. Of the samples, 53% were positive for campylobacters before tidal cover; this figure was significantly lower than the 64% recovered after tidal disposition. Nevertheless, no significant difference was found concerning the FIB numbers[23]. A more recent study had shown similar results, with a 100-fold increase in number of *E. coli* in the water under low-energy waves, while high-energy wave conditions produced a 1 000-fold increase in *E. coli* numbers[8].

Moreover, the presence of the wind in the coastal areas causes movement and transport of sand. This transport is influenced both by the characteristics of the wind and sand. Wind speed will have to reach at least 6 m per seconds at 30 cm from the ground in order to provoke movement of the sand. Particles with a diameter of about 0.1 mm are the first to be removed, whereas a violent wind is needed to remove larger particles. The percentage of the humidity in the particles of the sand influences the viability of the microorganisms and depends on the size of the particles, with the smallest ones containing more water. Therefore, the proper combination between the speed of the wind and the size of the particles create the appropriate conditions for the sand to move into the oral cavity[24].

Under the above conditions, it is obvious that sand itself might not constitute a hazard for the respiratory system because its particles are not <5 μm of aerodynamic diameter and consequently can not be inhaled and reach the lower respiratory tract to carry microorganisms and cause infection, contrary to silt and clay particles as mentioned before in this review.

8. Microorganisms isolated from the sand

8.1. Microorganisms of human origin

Enteric bacteria capable of causing gastroenteritis, like *Shigella* spp., have been isolated from sand samples.

In a study where the survival and adhesion ability of *Shigella* spp. in seawater microcosms was investigated; it was shown that the bacterium was able to adapt and survive under stressful conditions, such as temperature, osmotic stress and starvation. Adaptive survival mechanisms include a rapid change in the pattern of gene expression, often manifested physiologically, biochemically and structurally. This consideration is very important to explain the virulence of this bacterium[25].

Shigella spp. has been isolated from sand and water samples collected from the Gdansk gulf in Poland[26].

8.2. Microorganisms either of human or animal origin

8.2.1. Faecal index microorganisms

FIB are non-pathogenic microorganisms, which are used as indicators of faecal contamination. They are generally present in far greater numbers than pathogenic microorganisms in the gastrointestinal tract and are easy to isolate, indentify and enumerate in environmental samples. FIB include total coliforms, enterococci, bacteriophages and clostridia.

However, monitoring for these indicators is not always effective for determining when environmental samples are recently contaminated with sewage because FIB are able to regrow due to climate conditions conducive for regrowth and have mechanisms to survive the stressors frequently found in aquatic environments. The adapted strains may establish indigenous populations which thereafter are not indicative of recent contamination. Another problem is that FIB have different survival characteristics as compared to pathogens. They are more sensitive to inactivation by sunlight than viral and protozoan pathogens. Studies have also shown that even in the absence of any known sources of waste, FIB are consistently present. More recently, aquatic plants *Cladophora* and epilithic periphyton communities have also been identified as potential contributors to FIB regrowth[2]. Concerning the non-point sources, there is the question that whether the source of FIB may also be the source of pathogens causing the observed illness[27].

Various surveys often give different results on the number of microorganisms isolated from the sand. High numbers of thermotolerant coliforms and intestinal enterococci were isolated in beach sand along Taranto coastal waters in Italy[28]. Low numbers of FIB have been isolated in Tel Aviv and in Barcelona as well as from beaches near Rome[23,29]. FIB were also widespread at 53 California marine beaches, with *E. coli* and enterococci detected at 68% and 94% of the beaches surveyed, respectively; somatic coliphages and a bacteroidales human-specific faecal marker were detected at 43% and 13% of the beaches, respectively[22]. Moreover, in a study on 3 beaches in South Florida, total coliforms, bacteriophages and enterococci were isolated in higher concentrations from sand (2-23 fold in wet sand and 30-

460 fold in dry sand) in correlation to the adjacent bathing water[3]. Similar results have been documented by a study of 16 beaches of Sao Paulo State, Brazil, where higher levels of FIB and streptococci were found in dry sand during summer than in spring. Higher levels of bacteria in dry sand, that is not under the influence of the tides, may indicate that the main source of faecal contamination is not seawater, but instead the heavily polluted water of creeks and runoff. Lower levels in wet sand probably occur due to a "washing effect" of the seawater that seems to play more of a dilution role, than a contamination one[30]. Moreover, bacteria may survive better in sand because they are protected in biofilms. Sediments also provide osmoprotectors that negate the effects of high salinities. Sand grains provide as well a site for attachment and access to nutrients in the grain crevices[31]. Enterococci and *E. coli* have also been isolated at a rate of 91% and 62% respectively on 55 California beaches, while a high percentage of drug resistant enterococci (*Enterococcus faecalis* and *Enterococcus faecium*) have been isolated from 2 beaches in Brazil with a dominance of the resistant strains found on the most contaminated beach[32,33]. Another study, which examined the antibiotic resistance of bacterial stains, isolated from the sand samples of the beach located in the National Park of the southern Baltic sea coast, showed that, these microorganisms were most resistant in clindamycin and cefaclor, antibiotics which are commonly used in human and veterinary clinical practice. The highest antibiotic resistant was observed in bacteria isolated from the middle part of the beach and the dune, where human activity is higher, while the lowest antibiotic resistant was observed in bacteria from the sea. In marine waters and sediments two sources of antibiotic resistant bacteria can be identified, one from the bacteria originated from anthropogenic activities and the second from the indigenous marine bacteria. Environmental bacteria may, thus, play an important role as reservoirs of antibiotic resistance and differences in percentage of bacterial resistance to various antibiotics may reflect the history of antibiotic application[34].

C. perfringens can be found in many different habitats, such as the normal flora of human gastrointestinal tract, and environment, such as sewage and soil. On bathing beaches in Portugal it was isolated under varying tidal conditions[23].

Sources of the above mentioned microorganisms may be sewage that comes into contact with the beach, runoff from adjacent areas or residues of human and animal origin, particularly sea birds whereas an experiment showed that one faecal event from a gull could be spread over 3.1 m^2 of beach sand by pedestrians and natural transport mechanisms[2,3].

8.2.2. *Staphylococcus spp.*

According to the studies, staphylococci prevail in relation to the rest of the microbial flora in the sand, because it seems that the presence of staphylococci in sand is attributed to human activity and the keratinocytes that fall from the human body. Out of a total 85 species Gram-possitive grains that have been isolated from water and sand in 2 beaches in Chile, 31% were *Staphylococcus epidermidis*, 9% *Staphylococcus haemolyticus*, 24% *S. aureus* and 36% were identified as *Staphylococcus* spp. Interest arises from the fact that in the last few years methicillin-resistant *S. aureus* strains have been isolated from the seawater and sand[35].

According to a research of Papadakis *et al.*[36] in Attica area beaches (Greece), a higher number of staphylococci were isolated from the sand in correlation with the water and moreover a positive correlation was found between the concentration of the microorganisms, the density of the bathers, as well as the presence of yeasts of human origin.

8.2.3. Enteric bacteriae

In Israel, *C. jejuni* was isolated in small concentrations in samples of sand and water from a number of beaches, with the beach sand containing higher counts than adjacent shore waters. In the United Kingdom, *Campylobacter* spp. was isolated in 82 out of 182 beaches examined (45%), with a higher impact on wet sand in relation to the dry, although the percentage of isolation in dry sand was equal to 30%. The most often isolated species were *C. jejuni* and *Campylobacter lari*[23].

Salmonella spp. has been isolated from swimming beaches along the Gaza Strip and from 53 California marine beaches, at 15% of the tested beaches[22,37]. The same organism has been also isolated from one beach in Brazil, characterized as serotype agona. The presence of the bacteria in that area, could be attributed to a traditional crab dish, that is served in a restaurant at the beach. The crabs are caught and held on the beach prior to processing. It is known that many serotypes of *Salmonella* can survive for periods as long as several months when soil conditions are suitable[38]. Scientists at Liverpool, in collaboration with the Institute for Animal Health, have shown that *Salmonella* protect themselves inside amoeba. The research suggestd that amoeba may be a major source of *Salmonella* within the environment and could play a significant role in transmission of infection to man and animals[39].

In experiments carried out in freshwater sediment *Klebsiella pneumoniae* survived for weeks; nevertheless, it

has exhibited linear decay rates[1].

8.2.4. Viruses

In Romania, enteroviruses have been isolated during research carried out on the shores of the Black Sea with a seasonal distribution, with viruses absence both in sand and water samples during the non holiday period[23]. Additionally, samples taken during a water quality exceedance event at a Florida beach, impacted by non-point source pollution, were positive for the human polyomavirus in both sand and water[40].

8.2.5. Parasites

In a parasitological study of various national beaches in Guadeloupe, eggs of *Toxocara* spp. and *Ancylostoma* spp. were isolated[41]. In another study of two sand beaches in Marseilles, France, *Toxocara canis (T. canis)* was found to be the most common parasite, being present on average in 150 g of sand[23].

Contrary to above, in 226 samples in another study in Australia, neither eggs nor larvae of *T. canis* or other nematodes worms were isolated and in this study beaches frequently visited by dogs were included[23]; it was emphasized that older dogs are not a major risk for humans. In another study, from 16 beaches of São Paulo State, Brazil *Giardia lamblia*, *Entamoeba coli*, *Strongyloides stercoralis* and *Hymenolepis diminuta* in samples of dry sand have been found[30].

The parasitic arthropod *Tunga penetrans* (sand flea) is observed in endemic form in certain countries such as Central and South America, and has been inadvertently introduced by humans to sub-Saharan Africa and lives in the soil and the warm sandy soils, causing strong local inflammation at the point of attack on the skin, as well as bacterial superinfections. The "reservoir" of the parasite are dogs, cats and rats *etc.*[42].

In Cuba, microorganisms which belong to the family of Nocardioidaceae have been isolated from the sand on the islands of Jeju[43].

8.2.6. Fungi

It is known that fungi exist in the environment as saprophytes, but they may act as occasional pathogenic microorganisms especially to immunocompromised patients.

Yeasts are asociated with a variety of terrestrial and aquatic substrates. Sometimes they belong to specialised habitats and can be considered as indicators of pollution. The variety of yeasts found in the soil and seawater plays an important role in the medical pathology, causing cutaneous mycosis[38].

Fungi are often found in the sand and survive longer in correlation to other microorganisms due to the development of spores. It is estimated by researchers that their presence is related directly or indirectly to residues that leave the human body or the tidal influence[23].

In an *in vitro* study on four species of pathogenic fungi [*Trichosporon cutaneum, Candida albicans (C. albicans), Trichophyton mentagrophytes, Microsporum gypseum*], it was observed that they were able to survive in non-sterile sand for one month, while in a similar study 5 species of dermatophyton (*Epidermophyton floccosum, Microsporum canis, Microsporum gypseum, Trichophyton mentagrophytes, Trichophyton rubrum*) survived for 25 to 360 d[23].

In a research carried out in 3 beaches in Florida where 102 sand samples were examined, 21 species of fungi were detected through molecular techniques. The most frequently fungi detected were *C. albicans* and *Rhodotorula mucilaginosa*. A greater variety of species and higher concentration was found in dry sand in relation to the wet, while in general the density of the fungi was larger in the busiest beach[44]. *C. albicans* is an opportunist pathogen and has been associated with oral, vaginal and cutaneous infections in humans. This fact rises the need of sanitary quality standards for sand of beaches for recreational use[38]. In a more recent study, 33 beaches across Portugal were analyzed during a five year period in order to determine the presence of fungi; 60.4% were positive for the investigated fungal species. The majority belonged to *Candida* spp. Regarding dermatophytes the most predominant genus was *Trichophyton* spp. The number of colony forming units increased from June to August by the increase of beach users[20]. Last but not least, in a study in the Attica (Greece) area, Papadakis *et al.*[36] isolated 11 species of fungi, some of which were human pathogens.

8.3. Microorganisms of environmental origin

8.3.1. P. aeruginosa

P. aeruginosa is ubiquitous in water and soil. In nature the microorganism might be found in a biofilm attached to surface or substrate, or in a plaktonic form. *Pseudomonas* has very simple nutritional requirements and it is tolerant to a wide variety of physical conditions, including temperature and has a predilection for growth in moist environments, which is probably a reflection of its natural existence in soil and water. In experiments carried out in freshwater sediment *P. aeruginosa* survived for weeks; nevertheless, its has exhibited linear decay rates[1].

In a study along the coasts of Gaza Strip, *P. aeruginosa* was isolated from all samples of sand, thus generating the idea that it could be a good indicator of faecal contamination of the sand[37]. *P. aeruginosa* was isolated in Portugal, as well, in various swimming beaches under

different tidal conditions[23].

8.3.2. Vibrio spp.

On 3 beaches in South Florida, *Vibrio* spp. was isolated from the sea and sand, and in fact, at higher concentrations in the dry sand than in the wet one and sea water[23]. In the study on the Gaza Strip coast, *Vibrio* spp. was isolated in higher concentrations in the sand, despite the fact that only 10 g of sand was used while 1 L of sea water was examined. Statistically significant correlations were found between faecal coliforms and enterococci with *Vibrio* spp[37]. *V. parahaemolyticus* is a marine bacterium that requires salt and organic matter in the case of contaminated food, dose of 106 cells corresponded to a probability of disease of around 10%, and 104 around 1%. Below this the dose/response models diverged significantly.

In the United States oysters containing less than 10 000 *V. parahaemolyticus*/g are permitted to be sold; thus sand should not be a reason for concern. However, skin infection has been reported from a patient who was hurt, while opening oysters[45].

8.3.3. Aeromonas hydrophila

This microorganism has been isolated from sand in the Mediterranean coastal water at Tel Aviv.

It should be of special care for young children and immunocompromised adults, as it could cause gastroenteritis, cellulitis, myonecrosis and other infections[1].

8.3.4. Fungi

In Brazil in a study of 32 samples of sand from 2 beaches, 52 species of fungi were isolated, which were divided into 20 genera. The *Aspergillus* spp. and *Penicillium* spp. were the most often isolated species[46]. In the study of 33 beaches across Portugal during a five year period in order to determine the presence of yeasts, pathogenic fungi and dermatophytes *Aspergillus* spp., along with *Candida* spp. were the most frequent species found[20].

9. Infections caused by microorganisms isolated from sand

9.1. Gastroenteritis

A recent study of Henay *et al.* of 7 beaches in the United States found a positive correlation between the activities in the sand (*i.e.* digging and burying) and incidences of gastroeteritis, with burying in the sand more closely associated with the infections. Also, the proportion of those reported to have sand in their mouths was larger among those buried in the sand (40%) in comparison with those who dug in the sand (20%)[4].

In an earlier study conducted after visiting 3 beaches in North Florida, Bonilla *et al.* studied both the appearance of particular symptoms and concentrations of *E. coli*, enterococci and bacteriophages (somatic coliphage and F-specific coliphage) on these beaches. According to the research, a positive and dose dependant correlation was found between time spent on wet sand and the incidence of gastroenteritis (Table 3)[3].

9.2. Dermatitis

Dermatitis from *Ankylostoma* (cutaneous larva migrans) was developed in a group of travellers in Barbados with a higher frequency of infection in younger people and in those who less frequently used protective footwear while walking on the beach (Table 3)[47].

9.3. Infections of the eye

Studies on beach sand in Tenerife isolated *Acanthamoeba* spp. at a rate of 42%, while 100% of the isolated strains were potential pathogens[48]. In northern Florida *Acanthamoeba* spp. was isolated and more specifically, the genotype T4 in most cases, which is responsible for the majority of cases of *Acanthamoeba* keratitis (Table 3)[49].

10. Preventive actions

From what is known until today, it seems that the dominant risk from microorganisms to human health in bathing beaches and similar environments is the one resulting from contact with animal faeces and particularly those of young dogs (puppies). The latter often shed, with their faeces, eggs of the parasite *T. canis* which become infectious (*i.e.* contain the third stage larva) after 2 weeks in a favourable environment. Humans can be infected when they swallow the infectious eggs mainly through their

Table 3

Infections caused by microorganisms isolated from sand.

Infections	Microorganisms	Main points	References
Gastroenteritis	*E. coli*, enterococci, bacteriophages	Studies have revealed positive correlation between the activities in the sand and incidences of gastroenteritis	[3,4]
Skin infections	*Ankylostoma* spp.	Dermatitis from *Ankylostoma* (cutaneous larva migrans) developed in a group of travellers in Barbados	[47]
Eye infections	*Acanthamoeba* spp.	In Tenerife and in South Florida potential pathogen strains of *Acanthamoeba* spp. have been isolated	[48,49]

hands after contact with infected sand. In addition, dog is a "reservoir" of *Tunga penetrans* and the presence of an infected animal at the beach can infect the sand and respectively the beach users[23,42].

It would be possible, in this case, to set seasonal limitations for the accessibility of dogs, especially to frequently used beaches, or to place an obligation upon the owners to remove animal excreta. In some countries, particularly in resort areas, mechanical sand cleaning is a common practice that can eliminate the amount of organic matter and therefore reduce the further development of microorganisms. However, mechanical cleaning may disturb sand ecology. Studies that have been investigating the microbiological quality of the sand have shown that clear improvement was achieved by raising the general level of hygiene[23].

Chemical products are sometimes applied to sand for disinfection. Nevertheless, authorities in France have argued that there is not enough evidence to demonstrate the need and the efficiency of sand disinfection[23].

As other useful measures might be considered: washing hands before eating, the use of clean towels, sitting on the surface of the towel which has not come into contact with the sand, thorough washing and drying of the towels, the use of a new towel for the next visit to the beach, the avoidance of using straw beach mats, good personal hygiene, including shower before leaving the beach area, in order to achieve a faster removal of at least some part of the potential microbial load, the prohibition of animals' access to the beach and regular mechanical cleaning. The above actions can be considered as simple but important and effective in order to reduce microbial flora of the coast.

Another potential parameter to be considered is the human load, namely, the number of people using the beach. As it is mentioned above in the article, some researches point out the relation between the density of the beach users and the microbial sand load. Thus, correspondingly to guidelines for safe usage of swimming pools, it will probably be of some contribution, for studies to try to establish some limits on the number of "beach users", meaning a maximum number of bathers/beach users, per time and space unit; this might take into account also other parameters such as the size of the beach, the quality of the surface of the beach, whether containing sand- and the size of grains- or pebbles. Moreover, the cleanliness of the specific beach area might be considered, even in the cases where there is compliance of microbial quality of the bathing water of the respective beach with the international or national guidelines, as well as other factors.

11. Discussion

It was indicated that wet beach sand and sediment should be a part of epidemiological and microbiological studies, correlating recreational water quality with public health. Anyway, evidence to date is not enough to indicate that beach sand constitutes an infection hazard and that beach sands should be incorporated into a monitoring framework[1].

Although many studies have documented the presence of potential pathogens and pathogens in the sand of swimming areas worldwide, we lack basic information about their die-off rates, ability to persist and growth rate[1].

Radical differences in the various environments studied (subtropical beaches, coastal beaches, estuarine beaches, Great Lakes), climate, sand type, wave energy, point and non-point sources of pollution may contribute to bacterial concentrations, complicating comparing studies[1].

Another issue that should be further investigated is the eventual correlation between the speed and direction of the wind, in relation to the possibility that small particles of sand, silt or clay are swept up from the beach and into the oral cavity, with a risk of infection, especially among young children.

Additional factors like the control of the beach users' density and the implementation of mechanical cleaning may be capable to improve the microbial quality of the sand as well.

The activities that take place on the beach such as digging and burying in the sand as well as ingesting sand, particularly among children under 10 years old, seem to be common. Very few studies exist, which show that there is a positive correlation between gastroenteritis and exposure to beach sand. Future research is needed on this issue[4].

Moreover, complicating direct comparisons among studies, is the lack of a common method for detecting FIB in beach sand. As studies of sand begin to follow the same method, comparisons among them will become more meaningful.

An important issue, which is under investigation, is the use of FIB as microbiological markers of pollution, especially in the case of non-point sources. Epidemiological studies at beaches with non-point source pollution are fewer and have mixed success in correlating FIB abundance to bather health outcomes of enteric illness, respiratory and skin infections[1].

Taking into account the data of the National Survey on Recreation and the Environment during the period 1999-2000, over 44 percent of the civilian population aged 16

and over visit a beach. This translates to over 91.2 million people[50].

This fact brings us to the conclusion that if there is indeed a positive correlation between infections and microorganisms in sand, then this could be a important potential public health problem which could be prevented by reducing the microbial load.

Conflict of interest statement

We declare that we have no conflict of interest.

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