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The risk of contracting infectious diseases in public swimming pools. A review

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Summary. A review of pathogenic microorganisms presenting risk of infection in pool based artificial recreational water venues is extracted from the available scientific literature. The microorganisms are grouped both according to their way of spread and their survival and growth strategies and their characteristics relevant for the pool and spa based recreation are discussed. In order to put the proposed risks on a solid basis, among others a ten year excerpt of the waterborne disease statistics of the Centers for Disease Control and Prevention (CDC) is used throughout the article.

Key words: man-made water recreational environment, risk of infection, waterborne disease outbreaks, swimming pool, hot tub.

Riassunto *(Il rischio di contrarre malattie infettive nelle piscine pubbliche. Una rassegna)*. Viene presentata una sintesi della letteratura scientifica disponibile sui microorganismi patogeni potenzialmente infettivi, in ambienti acquatici ricreativi artificiali, come le piscine. I microorganismi sono raggruppati sia in base alle loro vie di diffusione che in base alle loro strategie di sopravvivenza e di crescita; successivamente vengono discusse le loro caratteristiche più importanti negli ambienti ricreativi considerati, piscine e terme. Per fornire adeguate e solide basi scientifiche ai tipi di rischi presentati, vengono analizzate, oltre ad altri documenti, le statistiche relative a dieci anni di osservazione di malattie legate all'acqua del Centers for Disease Control and Prevention (CDC).

Parole chiave: ambienti ricreativi acquatici artificiali, rischio di infezione, epidemie di malattie legate all'acqua, piscine, vasche da idromassaggio.

INTRODUCTION

Recreation has a substantial role in the life of ever increasing number of citizens in the world, and when choosing the scene for it, people tend to couple it with water. With evolving and advancing civilization, man-made water recreational environments are on the boom by offering health promotion and social benefits accompanied with increasing comfort and sophisticated services but also presenting to a certain extent of risk of physical, microbiological, or chemical nature. In the case of the so-called man-made water recreational environment, the extent of these risks can be reduced to a minimum by running the recreation facilities with the application of informed risk management measures [1].

According to the figures disclosed in the United States which can probably claim the greatest dimension of pool-based water recreation facilities [2], this is a solid basis for a huge market sector, and adverse health effects exerted in this environment to people or even a negative publicity may also have great economical consequences, including the direct price of health deprivation caused.

The main purpose of this paper is to review the relevant sources of infections in the man-made water recreation environment. It is neither aimed at producing a complete literature search nor dealing in depth with the methods of the risk management, but rather to summarise the most relevant pieces of information in the subject matter.

The weight of the possible adverse health outcomes of water recreation may be convincingly illustrated by specific waterborne outbreak surveillance data of the Centers for Disease Control and Prevention (CDC) – probably the largest, and continuously evolving national data collection in the subject. In spite of the considerable underestimation presumed by the compilers, 399 recreational water-borne outbreaks with more than 25 000 cases were registered in the United States in the interval from 1999 through 2008 [3-7]. Out of this number, 292 outbreaks (73.1%) with 23 800 cases (92.6% of all) were attributed to "treated recreational venues", *i.e.* pool, spa and similar facilities. Below, when looking into the aetiology of recreational water-borne infections, several reference will be made to this unique data pool [8] collected for a good deal of the population of the most developed part of the world.

Numerous infectious agents – bacteria, fungi, viruses and protozoa – may threaten the health or comfort of pool and spa users. Characterization of

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these pathogens according to the source and mode of transmission may be the key to the efficient way of risk management.

PATHOGENIC AGENTS OF FAECAL-ORAL SPREAD

Most classical and emerging waterborne pathogens are of faecal-oral in their mode of spread and thus share ill reputation in bathing and drinking water. In principle any microorganism capable of spreading by the faecal-oral mechanism can be involved in pool waterborne infection as water is an excellent vehicle for them. According to the source of infection, they can be both zoonotic and solely human pathogens with the direct source generally being another bather who shared the pool with the affected person, though much more rarely incidental cases and clusters can derive from infected animals which could access the pool or its close surrounding.

Most of the symptoms caused by them are enteric in nature but a couple of the microorganisms spreading this way can give rise to other types of diseases with serious generalised or localised neurologic, cardiologic respiratory or other manifestations or in other cases the primary, acute enteric disease may evolve into a secondary phase with a multitude of manifestations in other organs of the body [9].

The risk of contracting infection depends on the actual probability of the agent's incidence in the population usually visiting the pool and spa facilities. Pathogens which has never been detected or can be reasonably held exotic are out of the considerations targeting risk treatment measures while those that are frequently encountered in the population should be in the forefront of these measures.

Among the aspects rendering one or other pathogenic microorganism of possible incidence more or less likely to be actually getting involved in an outbreak are a couple of their inherent characteristics, like virulence and infectivity, rate and duration of post infectious shedding, environmental robustness and susceptibility to water disinfection.

Risk avoidance measures therefore should always be well informed about the incidence of relevant pathogens and their nature as to their aforementioned characteristics. If the risk connected to the most robust ones is appropriately addressed, that of others of similar nature but less vigour will more or less automatically be contained. Unlike in the case of non-treated natural bathing waters, the risk of infection by the management is addressed by actions targeting the removal of pathogens supposed to already be present in the water rather than preventing them in getting there. Nevertheless growing importance is given to measures which try to positively influence the users' hygiene behaviour in order to avoid introduction of the disease agents.

The source of these pathogens is almost invariably the ill or asymptomatic carrier who will shed them from the contaminated orifices and adjacent skin surfaces, although less frequently accidental faecal release (AFR) and vomit may be implicated [1]. Occasional cases may be caused by pools contaminated by infected animals having access to the pool.

The faecal-oral pathogens represent numerous bacteria, viruses and protozoa of widely different characteristic, with their infectivity (discussed mostly as attack rate) and their availability to water disinfection being consideration of utmost importance. Enteropathogenic bacteria tend to be more susceptible to disinfection and most of the outbreaks caused by them through pool water can more or less certainly attributed to the lack or gross inadequacy of the water treatment. On the other hand, in environments equipped with improved technology of pool sanitation, presently far most prevalent is the most robust pathogenic protozoon with high resistance to chlorine [10]. Given its outstanding epidemiological significance, *Cryptosporidium* will be discussed in a separate article.

Most publications on recreational water-borne outbreaks report cases from developed countries. It may be a true over-representation but there may be arguments for the outbreaks of this like being much more infrequent in the developing world. Enteric bacterial and viral infections' spread via public pool facilities could in principle present high risk in developing countries since the regulations and the accessible technical level may not raise real barrier to them. However, access to these facilities is more limited in these countries and thus the population low chance of to either shedding or contracting the pathogens via pools. For the developing countries, the major challenges in combating acute gastrointestinal infectious diseases are still the lack of safe drinking water and the low general hygiene standards.

Another, although scarcely documented factor may be the diverse likelihood of acquiring immunity to the infection by an enteric pathogen. An infection may confer protective immunity of very different duration (cfr. infection by hepatitis A virus and that by norovirus). Immunity is frequently limited in being protective by the genetic variability of the pathogenic agents – a characteristic *e.g.* of noroviruses [11]. Some of the emerging pathogens, most notably *Cryptosporidium*, have been shown to trigger an immune response [12] boosted during repeated infections thus rendering the risk of cryptosporidiosis less imminent. This effect may further be amplified by objective and subjective factors limiting persons suffering from self-limited gastro-intestinal infection in seeking medical assistance and being accessible for epidemiological investigation. In regions and countries where enteric pathogens of more severe health impact are highly endemic and even frequently epidemic, the effect of those having the greatest bearing in the developed world – like *Cryptosporidium* and norovirus may be much lesser. These factors may present to a certain extent explanation to the known big difference of

the cryptosporidiosis incidence even in the northern hemisphere.

To illustrate the distribution of prevalence of pathogenic agents in recreational waterborne outbreaks in a highly developed country, below frequent reference is made to the afore mentioned ten-year segment of the statistics compiled by the Waterborne Disease and Outbreak Surveillance System (WBDOSS) maintained by the CDC of the USA. During 1999-2008 an overall number of 166 outbreaks of acute gastro-intestinal infection (AGI) associated with treated recreational water venues (*i.e.* swimming and other pools and similar environments) were reported. This made out 72.5 percent of the total of 229 AGI outbreaks reported for all recreational waters, and 41.4% of the total of 399 recreational water-borne outbreaks, indicating the weight of risk for the pool and spa user community. While bacterial and viral pathogens were only responsible for 23 outbreaks (13.9%) and 663 (3.1%) cases respectively, the highly chlorine resistant *Cryptosporidium* dominated the statistics with 123 outbreaks (74.1%) and 18839 cases (90.2%). Another valuable source of information is an account of 12 years waterborne outbreaks in England and Wales between 1992 and 2003, reviewing 89 reported outbreaks of waterborne infectious intestinal disease affecting 4321 persons [13]. Swimming pools and similar facilities were implicated in 35 (39%) of these outbreaks affecting 762 persons. Unlike in the USA only parasitic protozoans (32 times *Cryptosporidium* and twice *Giardia* and *Cryptosporidium*) were implicated in the referred British swimming pool outbreaks.

Bacteria

Faecally derived bacterial pathogens, once dominating the waterborne outbreak reports seem to be represent an altogether low risk. Some of the ill-famed ones, like Salmonella species, including *S. typhi*, *S. paratyphi* seem to not at all being implicated in recreational water-borne outbreaks, though continue to be significant risk for drinking water outbreaks in the developing world [14, 15]. Rather than the survival capability in water, it is the infectious dose that may predispose an agent to be capable of effective spread and lending an attack rate necessary to trigger an outbreak in recreational waters. Recreational waterborne enteric pathogens stand out by extremely low infectious dose – typically in the range of 10 to 200 infective units.

The single most significant factor preventing enteric pathogen bacteria and viruses is the effective disinfection of the circulating pool water, as disinfection practices have been formulated just on the basis of experiences with them and reinforced by monitoring for indicator bacteria of similar sensitivity to disinfectants. Small wonder that all outbreaks linked to treated recreational waters (except for cryptosporidiosis) have been shown to be associated with gross treatment inadequacies and especially missing or ineffective disinfection. Venues which are not regulated, or regulation is not usually enforced are presenting typically a higher risk of infection. New types of man-made recreational water facilities, like interactive fountains or bio-ponds without disinfection have been shown to be emerging source of infection [7]. The pathogens still on the stage are those that stand out according to infectivity and still can be found circulating in the population even if at low prevalence.

The most important bacterial species of all seems to be *Escherichia coli*, which – though being the epitome of the commensal enteric bacterium – equipped with either of a couple of virulence factors is able to produce diseases of diverse severity from a short self limiting diarrhoea to frequently fatal haemolytic-uremic syndrome. It is the Shiga-Toxin producing (STEC) or verocytotoxin producing strains (VTEC) or by the widest known designation the enterohaemorrhagic *E. coli* (EHEC), that are almost exclusively implicated in waterborne outbreaks. Persons who have only diarrhoea usually recover completely but children under 5 years and the elderly are more frequently endangered by a complication called haemolytic uremic syndrome (HUS) – a life-threatening condition characterized by haemolytic anaemia and renal failure – that may occur in about 2-8% of infections. Secondary spread in man is common [16]. *E. coli* O157:[H7] is the most widely recognized VTEC serotype, but non-O157 EHEC strains are more common in most continental European countries and Australia. In contrast to the majority of diarrhogenic E coli strains known to be of solely human origin, *E. coli* O157 have been identified as zoonotic strain of bovine origin with several mechanisms of secondary spread, including waterborne events. The largest European outbreak in spring, 2011 was caused by *E. coli* O104 with predominantly foodborne origin, and mostly hit Germany with more than 4300 cases [17]. It is an indirect acclamation of the German level of pool hygiene and risk management that no secondary, pool-waterborne cases have been reported during the rather extended outbreak. EHEC is communicable for duration of fecal excretion (7-9 days) extending to up to 3 weeks in one third of children [18].

Recreational water related cases have sporadically been reported in the North America and in the United Kingdom and pool-borne ones are always linked to small paddling pools and other, small non-chlorinated facilities [19, 20]. A review of 20 years *E. coli* O157:H7 epidemiology in the USA revealed a total of 350 outbreaks with 31 (9%) recreational water related ones, a third of which linked to swimming pools [21].

Shigella has only humans and primates as natural host and causes in them symptoms from mild abdominal discomfort to dysentery, a serious condition characterized by cramps, tenesmus, diarrhea, fever. Some strains produce enterotoxin and shiga toxin that may cause haemolytic uremic syndrome similarly to some *E. coli* strains. Mucosal ulceration, dehydration and rectal bleeding can lead in neglected cases to death, as it happens according to the estimations of WHO in 108 000 cases a year

[22] mostly among children in the developing world. *Shigella flexneri* is another bacterium renowned as causing sequel like Reiter's syndrome in genetically disposed people [23]. The most frequent route of infection is via food or water contaminated with the faeces of shedding persons, by hand-to-mouth infection or via fomites and mechanical vectors, like houseflies. The incubation period is half to four days and the median duration of the disease is 5-7 days [24]. Though not more than 100 bacteria is enough for infection [25], water-related dysentery outbreaks more recently are rather rare in the developed countries. Infrequent outbreaks are reported from the USA, but most of them are caused by contaminated natural bathing waters sometimes in combination with other enteric pathogens [26]. Shigelloses linked to treated recreational venues have been reported in 7 occasions to the WBDOSS in the period of time chosen for analysis. All but one of them were caused by *S. sonnei* and involved 178 persons contracting the disease either in small, drain-and-fill type wading pools or residential pools, or when having fun with interactive fountains with missing or inadequate chlorination.

Campylobacter is a widespread zoonotic pathogen with the most prevalent species. *C. jejuni* is the estimated leading cause of acute infectious diarrhoea in most industrialised countries. It is overwhelmingly food-borne but certain outbreaks have been attributed to contamination of drinking water. *C. jejuni* infection is characterised by diarrhoea and is generally self-limited resolving in a couple of days (3-7 days). Serious complications of the infection may occur in about 1 out of 1000 infections with the most frequently occurring Guillain-Barré syndrome (see above) which have an onset several weeks after the diarrhoeal illness and lasts for several weeks to months.

Although infectivity of this bacterium seems to be somewhat lower than EHEC or Shigella, requiring about 10 000 cells to swallow [27], waterborne outbreaks occur relatively frequently [13, 28]. Poolwaterborne outbreaks on the other hand are rather rare, a reason for which may be that *C. jejuni* is more sensitive to chlorine than most other waterborne pathogens [29], rendering them more easily controllable by normal disinfection practices. The only reported pool waterborne cases can be traced to minor semi-public or private facilities, where even the minimum requirements of pool safety were unobserved. In a case of pool water mediated outbreak caused by *C. jejuni,* frequent presence of ducks was indicated by the investigators [3].

Viruses

In contrast to decades back in time, presently the viral outbreaks seem to outrange those of bacterial origin. It may be due to the modern molecular diagnostic techniques, but at least partially a true increase in incidence must have occurred because of changes in the epidemiological situation and the technology involved in the water recreational environment, favouring to the spread of more robust and contagious viruses. Adenoviruses, noroviruses, human enteroviruses hepatitis A and E virus and astroviruses are found most frequently in the literature as plausible viral causative agents in recreational water setting. For all of viral causative agents of waterborne outbreaks the major route of spread is the faecal-oral, though some adenovirus groups may infect by other mechanisms.

The etiological pattern of waterborne viral outbreaks is strikingly different from the general community outbreaks. Out of the three major virus groups – rotavirus, norovirus and adenovirus – causing the overwhelming majority of acute gastroenteritis diseases worldwide, only norovirus seems to have really significant role in the causation of recreational waterborne gastrointestinal diseases.

Adenovirus – a double-stranded DNA virus – has significant share in the burden of infections in the recreational water setting. Adenovirus comprises 54 human related serotypes [30], a third of which are incriminated in human diseases with gastrointestinal, respiratory, ocular, urinary and neurological manifestations [31]. Many adenovirus serotypes multiply in the small intestines and are shed in the faeces, but only 40 and 41 are unequivocally associated with gastroenteritis. Most adenovirus infections are mild or asymptomatic, except for those acquired in early childhood when it is second to rotavirus as a cause of childhood gastroenteritis. Human adenoviruses could be appropriate indicators of the presence of human viral pathogens in the environment [32] being more prevalent than enteroviruses due to their high stability in the environment [33]. Adenovirus 40 and 41 – found to be in the highest number of all studied adenovirus and enterovirus types in polluted water [32] – were postulated to have impact by drinking and recreational water outbreaks, but neither of them have been found in any of them to date, although frequent exposure is beyond doubt [34]. The only adenovirus outbreaks found in the literature are incidents of pharyngoconjunctival fever, caused by either of serotypes 3, 4, 7 or 7A, the most prevalent adenoviral agents of upper respiratory tract infections. Notably almost all of these waterborne outbreaks have been linked to pool setting calling the attention to specific routes of infection by them. Beyond the typical route of ingestion water contaminated with faeces or other excreta containing viable virus particle, simple rinsing of the throat or conjunctiva, exposure to airborne droplets of contaminated spray, or even direct airborne person to person infection in the crowded setting can be endorsed. Of the reviewed 55 recreational waterborne viral outbreaks selected by scientifically sound inclusion criteria from the literature of 55 years between 1951 through 2006 [35], 13 were caused by adenovirus and 11 of the latter was linked to pools, once qualifying adenoviruses the number one viral agent in pools. The reviewers hypothesis of the decline of incidence of pharyngoconjunctival fever outbreaks

over the decades examined seems to be supported by the data retrieved by the WBDOSS in the USA from 1999 to 2008, when none of the outbreaks linked to treated recreational water venues were caused by adenoviruses. Since then another case with 59 affected children caused by human adenovirus serogroup 4 was published in Spain [36].

Two further virus groups can be highlighted as those causing the highest number of enteric infections worldwide. Rotaviruses – a double stranded RNA virus group – are the leading causative agent of severe diarrhoea among children with a very high death toll in the poorest part of the world, having killed 527 000 children under 5 year age in 2004 [37]. The incidence of rotavirus infection is similar in developing and developed countries and in the prevaccination era 95% of the children were estimated to experience rotavirus infection by the age of 5. Reinfection can occur at any age and subsequent infections are generally less severe due to progressive immune protection [38], with mostly subclinical infection in adults.

In contrast to rotavirus, norovirus (single stranded, small-round structured RNA viruses; on earlier names: Norwalk virus or calicivirus) may cause gastroenteritis in any age and in fact are the most prevalent cause of it in the world with an estimated one billion case of acute diarrhoea a year. In the USA an estimated number of 21 million illness, 70 000 hospitalization and 800 death, is the toll of norovirus [39]. It mainly causes 1-3 day long self-limiting diarrhoea after 24-48 hours of infection, but severe symptoms may be manifected in early childhood and in elderly persons. Around 30% of infections from norovirus are asymptomatic, but the infected persons shed the virus. It is highly contagious, and less than twenty virus particles can cause an infection [40]. Although both rotavirus and norovirus can be implicated in water contamination incidents leading to waterborne outbreaks, there is great difference between their real involvement. While norovirus is found to play significant role in waterborne outbreaks [41], evidence for rotavirus causing such outbreaks is rather sparse [42, 43], though it was occasionally found together with other pathogens in case of gross contamination [44]. The difference is even more marked for recreational waters and especially for pools and similar environments. Indeed, rotavirus is missing from the review of Sinclair, *et al* [35]. Norovirus outbreaks are however abundantly demonstrated in the literature since the early detection of this virus. Out of 55 definitely recreational waterborne outbreaks studied between 1951 and 2006, 25 (45.5%) was found to be caused by norovirus giving the highest contribution out of the six virus groups considered. Out of these outbreaks 7 (28%) was linked to pool water. Rotavirus was not found by WBDOSS among causative agents in poolwater in the USA between 1999 and 2008, while norovirus caused 9 outbreaks (n.b. there is a considerable overlap in both cited pool of outbreaks). An explanation for this can be the distinct pattern of immunity between both types of viruses. As above pointed out, protective immunity against rotavirus infection is universal after the first couple of life years in contrast to norovirus which does not induce lasting immunity [45] among others because of the great genetic variability of the genome of the virus.

Astrovirus, another small single stranded RNA virus is ranking the fourth most common known cause of viral gastroenteritis. Infection is rather common already in the early ages of life, and generally causes very mild self limiting diarrhoea or even more frequently remains asymptomatic. Although significant role is attributed to contaminated water in its spread, only one waterborne astroviral outbreaks have so far been revealed [13]. In the peer reviewed literature the single mentioning of astrovirus in association with recreational water was published in Finland, where it was found both in the water of an outdoor wading pool and the patients' stool specimens together with norovirus [46].

Another couple of viruses of predominantly faecal-oral route of infection are occasionally also implicated in recreational pool waterborne outbreaks but they are distinct by causing diverse clinical manifestations other than symptoms of acute gastroenteritis. A group of small single stranded RNA viruses of the Picornaviridae family are the enteroviruses classified into four groups for A through D comprising poliovirus, coxsackievirus A and B (both with several serotypes), echovirus (with 6 serotypes) and enterovirus 71. From the present review, Poliovirus, once having caused the most feared outbreaks of poliomyelitis can be excluded on the basis of its almost complete eradication. Though most of these viruses are entering the body via ingestion, multiply in the intestinal tract and are shed with faeces, gastrointestinal illness is the least characteristic disease they can cause. The wide array of symptoms they cause, extend from neurological, through cardial, conjunctival, respiratory and dermatological manifestations, although most infections result in mild or asymptomatic illness, with the highest incidence in children. The incubation period is usually less than 5 days and the infected persons frequently shed the virus before the symptoms emerge and continue to shed until several weeks after recovery. There are global or at least continental epidemiological trends with the emergence of one or more serotypes of enterovirus and given their great contagiosity, the high cross-infection rate of the actual variants quickly establishes dominating prevalence of them [47]. Out of the 55 recreational waterborne outbreaks referred by Sinclair *et al.* [35] 12 was caused by enteroviruses (coxsackie and echoviruses) and 7 of them (all caused by echovirus types) could be linked to swimming pools. The ten year surveillance data of the CDC WBDOSS refer only one enterovirus outbreak (echovirus 9), which is also covered by the above referred review.

Two further viruses both causing hepatitis in infected persons are finally considered as pathogens possibly causing recreational water outbreaks. Hepatitis A virus (*Picornaviridae* family) and hepatitis E virus

(*Hepeviridae* family) are unrelated though the infection by both of them induces similar, generally mild symptoms of hepatitis, or even more frequently, particularly at childhood infections, remains symptomless. Hepatitis viruses are very contagious and their spread has a distinct pattern depending on the general hygiene circumstances. Waterborne spread of both viruses are well documented, with major drinking waterborne outbreaks of hepatitis E in Asia [48-50]. Although several authors isolated Hepatitis A virus from surface waters, which were used for recreation (*e.g.* [51]), outbreaks related to recreational water are relatively rarely reported. Long incubation period may contribute to the difficulties the detection of these outbreaks. Both the reviews of Sinclair *et al.* [35] and Pond [52] cites three credible pool-related outbreaks worldwide, and none have been identified by the CDC WBOSS team between 1999 and 2008.

Protozoa

In addition to *Cryptosporidium*, which is the most important cause of waterborne outbreaks in the developed part of the world, the only significant protozoan agent to cause recreational waterborne outbreaks is *Giardia duodenalis*, a unicellular parasite that infects humans and a range of wild and domestic animals. Giardia is capable of surviving long environmental exposure in the form of resistant cyst and giardiasis is the most frequent intestinal disease worldwide caused by a protozoan estimated to cause about 300 million cases a year [53]. Waterborne spread is one of the most significant mechanisms of Giardia infections [54]. Very few cysts can establish infection, though the majority of infected persons remain asymptomatic cyst shedder and most of the rest will experience a self limited acute diarrhoea lasting 1-3 weeks [28]. Symptomatic disease ranges from mild diarrhoea to a severe malabsorption syndrome [55]. Asymptomatic cyst passage can last as long as six months [56]. Giardia is relatively resistant to disinfection processes, and its risk is confirmed by several poolborne outbreaks and surveillance data rewieved by Pond [52]. In the years 1999-2008 six giardiasis outbreaks with 216 infected persons linked to pools were reported to the CDC WBDOSS. In half of these outbreaks mixed pathogenesis with cryptosporidia were revealed.

Microsporidia, a large phylum of highly specialised obligate intracellular parasites with more than 700 species are infecting a range of divergent hosts from insects to mammals including humans. Once thought to be protozoans, more and more authors bring reasons for their identity as fungi [57]. They are considered as the fourth most prevalent protozoans causing diarrhoeal disease worldwide (with an estimated 30 to 70% infection ranges [58]), though primarily immunocompromised persons are at highest risk of severe disease. The infection with Microsporidia can result not only in enteric disease but a number of other organs, from the cornea to the kidney and central nervous system can be attacked. The most frequently found species involved in human pathogenic process are *Enterocytozoon bieneusi* and *Encephalitozoon intestinalis*. Although only few data are available to confirm their connection with pool waterborne infections, both their worldwide distribution in water also for different human uses as reviewed by Pond [52] and occurrence as highly resistant spores capable to survive even several years in wet environment, predispose them to be considered as emerging pathogens of this sort.

PATHOGENIC AGENTS WITH OTHER THAN FAECAL-ORAL SPREAD

There are several groups of microbes that can spread by other than faecal-oral route and cause diverse diseases or conditions other than gastrointestinal. Some bacteria are capable to exist both in saprophytic and parasitic life-cycles, and man-made recreational water environment offers appropriate niches where they thrive in microbial biofilm communities until opportunity opens for entering a parasitic cycle in humans.

For a couple of other organisms recreational water venues provide merely sites of passive transfer from carriers to new hosts when opportunity opens to find the way of entering it. They can incidentally infect susceptible hosts mostly by way of their contacting contaminated surfaces. Mediating environment can be physical objects in and around the pool and its facilities (steps, rails, pool-bottom and walls), fomites like slides, benches, sunbeds and surfaces of the ancillary facilities (toilets, locker rooms, etc.).

Biofilm mediated organisms

One of the most frequently reported pathogen in the man-made recreational setting and the one requiring doubtless the highest death toll is Legionella.

The Legionella genus consisting 57 species [59] of an ubiquitous aquatic organism [60] has recently been identified as causative agent of actual and earlier outbreaks of respiratory diseases, commonly called legionelloses. The gravest syndrome, legionnaires' disease – a severe pneumonia – can attack anyone but has the highest death toll in elderly or immunocompromised persons and those suffering underlying conditions like diabetes, alcoholism and other chronic diseases where mortality can be as high as 30-40%. The type species called *L. pneumophila*, and first of all its serotype 1 (out of 16 identified to date) is responsible for far the most pneumonia cases with further 23 species associated with human disease [61]. Another form of legionellosis, a non-specific upper respiratory set of symptoms (Pontiac fever) is self limiting and short duration febrile flue-like illness that has been associated with exposure to *L. pneumophila*. In contrast to legionnaires' disease, Pontiac fever has a very high attack rate affecting up to 95% of the exposed persons [62]. Incubation period is 2-10 days in case of the legionnaires' disease and a few hours to 2 days in Pontiac fever [63].

Legionella raised scientific interest not only because of being a dangerous emerging pathogen, but also by its particular "dual life cycle", offering experimental model for molecular biological research into the bacterial intracellular differentiation. Legionella is ubiquitous in natural water bodies and wet soil and its success in survival and competition is ensured by its ability to infect protozoa and proliferate in them – the parasitic mechanism also utilized in the pathomechanism of legionnaries' disease as human pathogen when it parasitizes the alveolar macrophages. Its persistence in man made water environments is linked to biofilm communities [64] where it can establish itself and at a temperature range of about 25-45 °C has a solid selective advantage in the competition [65]. Symbiotic or parasitic growth in free-living protozoans have been shown to yield further benefits to Legionella, not only by conferring protection against harsh environments like biocids, but also imparting higher invasiveness and human pathogenicity [66]. There is though some controversion in the scientific literature whether Legionella can multiply in biofilm without having recourse to parasitic multiplication in amoebae [67], but there is no doubt about the significant selective benefits of this mechanism for the organism [68].

Transmission of Legionella takes place via aerosols containing droplets of diameter of less than 5 μm. The infective dose is small and the circumstances of exposure have a definitive role ensuring it arrives to the site of entry. There are a couple of anthropogenic water-based environments that frequently serve as a niche of Legionella multiplication and a basis for subsequent human exposure. Next to evaporative cooling systems and extended hot water networks in large buildings, pool and spa setting is the third most significant setup where the most infection occurs. All three share characteristics that support Legionella and the associated biofilm organisms in proliferating and reaching human hosts: large, relatively warm, stagnant or slowly circulating water bodies in contact with extended surface and carrying dissolved and suspended nutrients. The biofilm once established and matured, offers excellent protection against adverse environmental effects, and trough sloughing gives rise to suspended cells that find way through specific sites of exposure to the host. This provides the key to measures of risk avoidance in the case of whirlpools, spa pools or hot tubs and similar facilities that are far the most frequent risk sources in recreational setting, where the establishment of the biofilm is the key factor rather than the physical circumstances of the exposure. Exposure and establishment of infection does not necessarily mean disease: most people will not develop clinical symptoms even in the evidence of exposure [69]. Due to the intrinsic characteristics of the spread of the legionnaires' disease, it mostly occurs as sporadic cases and small clusters, although large outbreaks also occur – mostly in conjunction with contaminated cooling towers [70].

There are abundant sources in the scientific literature proving the great burden of legionellosis diseases [1, 27, 61, 69, 70]. In spite of the great efforts of surveillance, the disease remains largely underreported due to the difficulties of revealing sporadic cases, and because its symptoms are rather unspecific and respond well in most cases without exact diagnose to antibiotic treatment. The incidence of reported cases of legionellosis in the USA in 2009 was 10.8 [71] and in Europe (for 25 EU member states, Norway and Iceland) 11.2 per million of population [72]. The true incidence however is estimated 100 per million. Out of this relatively large number of cases in Europe, only 254 pertained to 101 clusters. As environmental investigation is generally lacking, the mode of infection beyond its being travel associated cannot be resolved, and there are no data in the annual reports on the proportion of cases and clusters caused by pool and spa facilities. They present however principal risk during travel and this may have extended with the growing popularity of such facilities in hotels and other sites of accommodation. The most recent cluster of travel associated legionellosis in Calpe, Spain with 40 cases during more than half a year turned out to have been caused by a spa pool [73].

In the United States, 24 pool-linked outbreaks were reported to the CDC WBDOSS, in the reviewed time span, 5 of which was outbreak of Pontiac fever alone or mixed with cases of legionnaires' disease. Pond reviewed altogether 30 outbreaks of legionellosis linked to recreational waters and found that the greatest risk of infection has been linked to either thermal spas where no disinfection is allowed or hot tubs/spa pools [52]. Display hot tubs operated without disinfection have caused severe outbreaks on several occasion, evidencing that the risk of infection is high without immersing in the contaminated water [74-76]. Although Legionella bacteria were incidentally isolated from swimming pools, they have never been identified to cause legionellosis, except for cases linked to poolside showers [77]. As a specific subset of travel associated cases, spa pools on ships are also frequently implicated in causing legionnaires' disease [78]. When looking at the circumstances that lead to infections of Legionella in pool and spa facilities, mismanagement and first of all flawed disinfection seems to be the major triggering factor [63] calling for more efforts of control and supervision.

Pseudomonas aeruginosa – the most frequently referred to opportunistic pathogen bacterium in connection with the pool and spa environment – is a nutritionally highly versatile, ubiquitous aquatic bacterium capable of adapting to various environmental conditions including water, vegetation, soil and various niches of the human body [1, 79]. Although not belonging to the typical resident skin microflora and infrequently colonizing (2.6 to 24% depending on the location tested) in non-hospitalized adults [80], *P. aeruginosa*, may yet be considered a normal constituent of the human natural microflora [81]. Besides silent colonisation, as a tough opportunistic pathogen armed with a number of virulence factors and

antibiotic resistance [82-84], it can overtly infect humans mostly with compromised immune system but not infrequently also healthy ones [85, 86]. The most prevalent location of infection originated in pool and spa environment in healthy people is the skin and especially that of the outer ear canal, with related clinical symptoms like swimmer's ear (otitis externa) and hot tub rash (folliculitis). Reports in the peer reviewed and grey literature give extensive description on outbreaks of otitis externa, folliculitis and/or dermatitis rash with variable severity and duration of up to > 6 weeks [87, 88]. In the interval between 1999 and 2008 a total of 52 outbreaks of dermatological infection by *P. aeruginosa* were reported with 955 cases. Thus *P. aeruginosa* is ranking as the second most prevalent pathogen after *Cryptosporidium* in the pool and spa environment in the USA. The true incidence of whirlpool-associated P. folliculitis is difficult to determine because the symptoms are often mild and self limited, and the patients frequently do not seek medical attention [89].

Otitis externa is characterized by inflammation of the external auditory canal. Factors increasing the risk of otitis externa related to water exposure include amount of time spent in the water prior to the infection, a record of previous ear infections and repeated exposure to water by slackening the protective wax coating of the outer ear canal [90, 91]. Another specific manifestation called hot foot syndrome – a clinically distinct painful erythematous plantar skin eruption – was found to be due to high densities of *P. aeruginosa* in a pool with an abrasive flooring [92]. Occasionally, *Pseudomonas* infection may give rise to more serious consequences like corneal ulcer or wound infection, respiratory system diseases and urinary tract infections [93]. Faecal and non-faecal shedding from humans is suggested to be the major source of *P. aeruginosa*, but in fact the importance of the primary source is often outdone by the conditions favourable to its growth in attached biofilms in and around the pools. Such locations may be various pool structures (linings, decks, drains, filters) and surrounding objects and fomites from the benches to towels and children's toys [94]. The connection of the abundance of *P. aeruginosa* sources and occurrences with the intermittent incidence of documented health damages and outbreaks is still not well understood. Rather then a classic dose-response relationship which would indicate a highly variable risk to healthy individuals, a variety of related factors like the contact time, the biotype and virulence of the implicated strain and a couple of personal conditions of the bathers (like time spent and repeated exposures in water, the water temperature, history of earlier infections, etc.) has heavy impact on the probability of getting ill from a *P. aeruginosa* infection [94]. Several authors assess the circumstances that lead to outbreaks and they agree in finding inadequate or completely missing disinfection the main factor [7, 91]. The effect of chlorine repeatedly dosed in the form of cyanurates may largely decrease due to cyanurate-lock – a contraindication of using these compounds in whirlpool spas. Sometimes outbreaks of *P. aeruginosa* (and also Legionella) infection are epidemiologically linked to both the swimming pool and the spa pool of the facility [7]. Experiences of the CDC WBOSS team prove contributing conditons like exposure occurring in a hotel/motel setting, in which spa operation is not a full-time job and exposure in the context of a group event when crowding may quickly deplete disinfectant levels.

Several non-tuberculotic *Mycobacterium* species (like the *M. avium-intracellulare Complex /MAC/, M. chelonei, M. fortuitum, M. gordonae, M. kansasii, M. marinum*) were detected in various water environments and described to be involved in various pathologic processes [95]. The environmental mycobacteria are biofilm-associated [96] and are proposed to profit from endosymbiotic relationship with protozoa where they may enter into more virulent status [97]. In the majority of cases waterborne – and among them pool-related – mycobacterial infections present a risk for persons of compromised immunity or other underlying condition, like open wounds, cystic fibrose, etc. [98, 99]. Atypical mycobacteria have also been frequently isolated from healthy individuals [100] and are frequently called "leisure-time pathogen", referring to the fact that the infection is usually associated to activity during water recreation. The most frequent form of the infection in healthy individuals is a self-limiting form of skin granuloma on prevalent locations like elbow, knee or wrist and may also occur in epidemic form involving several users of swimming pools. The infection is frequently linked with minor skin abrasions. First of all *M. marinum* is associated with swimming pool granuloma outbreaks [101]. Respiratory system disorders (hypersensitivity pneumonitis, hot tub lung) have also been associated with mycobacteria, first of all with MAC mostly in connection to the use of poorly maintained and non-disinfected spa pools [102, 103].

Several types of unicellular protozoa (free living amoebae, FLA) have been described that thrive in abundance in natural or artificial water environments but are also able to invade at variable ports human hosts and cause severe syndromes. They are all characterised by favouring higher ambient temperatures close to that of the human body and by changing lifecycles adjusted to the circumstances. The FLA of the worst fame is *Naegleria fowleri* which can inflict primary amoebic meningoencephalitis (PAM), an almost always fatal condition. *N. fowleri* is often found in warm waters used for recreation and curative purposes, but less frequently in treated recreational waters. Naegleria can exist in three different appearances: its dangerous amoeboid trophozoite form is its reproductive (and frequently invasive) format. Transitionally it lives as a motile flagellate or in unfavourable conditions it stays inactive as an environmentally resistant cyst capable

to hatch (excyst) whenever circumstances turn favourable for feeding and reproduction. The trophozoite form enters the body of the host via the nasal mucosa – frequently under hydraulic forces when jumping into the water or diving – and invades the brain via the olfactory nerve. The disease is generally fatal about 3 to 10 days after the onset of the symptoms which in turn follows the infection in 7-10 days [104]. The number of cases seems to grow year by year, and though most of them are linked to natural waters (*e.g.* small warm ponds) pool-associated cases are also published. A cluster of cases happened about 50 years ago in a single indoor pool in Northern Bohemia where 16 young patients died in fulminant PAM caused most probably by *N. fowleri* [105]. Since than several hundred cases were identified which were mostly associated to natural waters but incidentally thermal pools have also been involved [104].

Another protozoan frequently described as water associated pathogenic agent is Acanthamoeba. This FLA is rather often found in recreational waters and among them in swimming pools [106], and is responsible for about 5% of the contact lens related microbial keratitis cases. Though most cases are linked to using unsterilized contact lens fluids, swimming in contact lens is described as a risk factor to be avoided [107]. Another serious disease caused by some genotypes of Acanthamoeba is the granulomatous amoebic encephalitis (GAE) a subacute or chronic but invariably fatal brain infection. The route of infection is thought to be by inhalation of amoebae or introduction through skin lesions. Unlike with amoebic keratitis, victims of GAE are mostly persons with conditions compromising the immune system, though GAE cases of immunocompetent children and adults were also described. In contrast to N. fowleri, no clearly swimming pool related GAE cases have been reported to date though its potential risk is apparent in the presence of FLA of proven pathogenicity [108].

Biofilm related conditions not coupled with a specific microorganism

Respiratory symptoms impossible to clearly link to a specific microbe are incidentally associated with pool and spa setting and are attributed to allergenic reaction to inhaled bacterial endotoxins. Outbreaks of granulomatous pneumonitis among lifeguards have been associated to occupational exposure to aerosol generated by indoor pool-related water features with high numbers of bacteria (*Pseudomonas aeruginosa* and others) in the water and high concentrations of endotoxin [79, 109]. Beyond published cases, anecdotal incidents also crop up from owners of residential whirlpools. The cases are always associated to gross neglect in maintenance and missing disinfection with consequential very high bacterial counts in the water of the whirlpool. These phenomena are also clearly associated to pool-related biofilms and their prevention needs measures just like risk management for infections caused by specific biofilm microorganisms.

Infections by microorganisms of other than faecal-oral spread by way of passive transmission

For a complete overview of microbiological risks in the man-made recreational water installations, consideration should be given to a diverse group of non-faecally derived microorganisms that are carried by persons – or incidentally by animals – with or without symptoms of various infections and shed into the water or onto surfaces of objects in the pool and spa facilities and may infect susceptible hosts by plain encounter. They present temporary nuisance rather than serious disease, and are generally sparsely referred by the scientific literature but still may present a considerable social and health burden. In contrast to the majority of these, Leptospira, the only relevant zoonotic agent that may be accidentally introduced by an infected animal into a man made recreational water can cause serious, and even fatal disease. The Leptospira genus consists of several saprophytic, intermediate and pathogenic species, and out of them *L. interrogans* – and first of all its serovar *L. icterohaemorrhagiae* – causes the most severe form of leptospirosis (Weil's disease). Leptospirosis has rather variable clinical manifestations from a mild flu-like course to the severe, often fatal icteric form characterised by kidney and liver failure. The source of the infection is always an animal host, in case of *L. icterrohaemorrhagiae* infections most probably rats, that excretes the bacteria with it urine. Leptospira is rather sensitive to environmental stress but on the other hand extremely infective: a very small number of bacteria (1 to 10 cells) can establish the infection by various ways of entry. It can penetrate the skin on small abrasions and the mucous membranes if swallowed, inhaled, or contacted (*e.g.* conjunctiva). There are several reports of waterborne leptospirosis outbreaks but almost invariably in conjunction with bathing in natural freshwaters. Pool water related cases are probably very rare; two outbreaks reported to date are associated with non-disinfected swimming pools [1].

An opportunistic pathogenic bacterium, *Staphylococcus aureus*, is frequently found as member of the microflora of skin or nasal mucosa of healthy individuals and is invariably shed when immersing into the pool water [110]. Its presence in the water in high numbers may be a consequence of crowding and inadequate disinfection and may cause skin infections (rashes, impetigo, otitis externa) wound infections, conjunctivitis, etc. It is sometimes involved in outbreaks (in the above referred ten years, 2 were reported to the CDC WBDOSS team), but it is rather infrequent. Growing concern is attributed to emerging methicillin resistant strains [111]. Density of coagulase positive *staphylococci* in water have been proposed as an indicator with relevance for both the bather load and the effective disinfection [112].

There is only anecdotal evidence of transmission via water of swimming pools or other pool types of *Trichomonas vaginalis*, an unicellular protozoan known as causative agent of trichomoniasis, a rather prevalent sexually transmitted disease. It causes mild, but without treatment lasting nuisance symptoms in infected women, though the proportion of asymptomatic infections is about 70%. The possibility to contract infection this way is controversial. Trichomonas have been shown to survive and remain viable for several hours in swimming pool water [113] but others argue against it claiming that it loses infectivity very quickly in water [114].

A frequently encountered viral skin lesion, *molluscum contagiosum* (sometimes called water wart or swimming pool wart) is caused by *molluscum contagiosum virus* (MCV), a member of the family of the double stranded DNA *Poxviridae*. MCV is infecting only humans and spreads mostly via direct skin-to-skin contact but indirect spread via fomites is also frequently reported. The infection is most prevalent in children under 10 years and is occasionally proposed to be associated to the use of swimming pools [115]. Researchers who have investigated this idea think it is more likely the virus is spread by sharing towels and other items around a pool or sauna than through water [116]. The infection is presented after 2 to 6 week incubation period on the trunk, legs or arms by round lesions of 1-5 millimeter in diameter which are flesh-colored, dome-shaped, and pearly in appearance. An individual lesion lasts generally 6 to 8 weeks, but it can spread from one skin area to others via autoinoculation which considerably may prolong the process up to 6-8 months.

Another relatively innocuous dermal nuisance, *verruca plantaris* or plantar wart is caused by a human *papillomavirus*. Plantar warts are thought to affect 7-12% of the population with higher prevalence in school aged children. A plantar wart is a benign tumour that appears on the sole of the foot and typically resembles a cauliflower, with tiny haemorrhages under the skin in the centre. It may be painful under pressure when walking or standing. Plantar warts are usually acquired via direct physical contact during barefoot activities on surfaces like shower and changing room floors contaminated with infected skin fragments though not necessarily in association with pool and spa facilities [117], and they are not at all transmitted via pool or hot tub waters. Also, it may spread through autoinoculation. The virus is extremely contagious as it is able to survive several months on dry surfaces until encountering a host. The incubation period ranges from 1-6 months; however, latency periods of up to 3 years or more are suspected. Plantar warts are usually self-limiting, but treatment is generally recommended in order to reduce symptoms and to prevent transmission.

Rather prevalent skin infections proposed to be more widespread among pool and spa patrons are some types of dermal mycoses, caused by *Epidermophyton floccosum* and several species of Trichophyton causing superficial infection of the keratinized areas of the body (skin, hair and fingernails). The far most frequent skin infection in association with pool and spa establishments is tinea pedis or by popular label athlete's foot, generally caused by *T. rubrum* or *T. mentagrophytes*. These can survive on a variety of surfaces and objects, like sand, floors, shower stalls, clothing, and hairbrushes) and are transmitted via wet surfaces where people walk barefoot or by contaminated fomites. Athlete's foot causes scaling, flaking, and itching of the affected skin. In more complicated case, blisters and cracked skin occurs, with pain, swelling, and inflammation of the exposed soft tissues and possibly leading to secondary bacterial infection. Tinea pedis most often manifests between the toes but can infect other parts of the body (called then tinea corporis, tinea cruris, etc.). Depending on the species, dermatophytes can survive up to twenty months on skin scales at room temperature. The role of communal bathing places such as indoor swimming pools or other bathes in the spread of tinea pedis has been well established [118].

Swimming pool related dermatomycoses can only be controlled informed hygiene management measures and by concurrent education of people for using pool establishment consciously about the risks of these and the way of evading them. This is also true in association with all other infections mentioned above supplemented with public health efforts to maintain surveillance for early detection of emerging risks and to bring up and support new, effective means of management.

Conflict of interest statement

There are no potential conflicts of interest or any financial or personal relationships with other people or organizations that could inappropriately bias conduct and findings of this study.

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