

Waterborne microorganisms and biofilms related to hospital infections: strategies for prevention and control in healthcare facilities

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ABSTRACT

Water is the main stimulus for the development of microorganisms, and its flow has an important role in the spreading of contaminants. In hospitals, the water distribution system requires special attention since it can be a source of pathogens, including those in the form of biofilms often correlated with resistance of microorganisms to various treatments. In this paper, information relevant to cases of nosocomial infections involving water circuits as a source of contaminants is compiled, with emphasis on the importance of microbiological control strategies to prevent the installation, spreading and growth of microorganisms in hospitals. An overview of the worldwide situation is provided, with emphasis on Brazilian hospitals. Different approaches normally used to control the occurrence of nosocomial infections due to waterborne contaminants are analyzed, and the use of the polysaccharide chitosan for this specific application is briefly discussed.

Key words | antimicrobial agents, chitosan, hospital infection, microbial contamination, water contaminants

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INTRODUCTION

A hospital infection can be defined as any infection acquired after patient admission and manifested during hospitalization or after patient release, being then related to hospitalization or hospital procedures. A hospital infection can be also defined as a localized or systemic condition resulting from an adverse reaction to the presence of an infectious agent(s) or its toxin(s) that was not present on admission to the acute care facility. Commonly used synonyms of hospital infection include the terms nosocomial or healthcare-associated infection (HAI).

The transmission of microorganisms responsible for nosocomial infections is a serious and recurrent public health problem, affecting both developing and developed countries. As an example, in the USA around 2 million healthcare-associated infections occur yearly, causing approximately 90,000 deaths and costing close to \$4.5 billion in excess healthcare (Ecker & Carroll 2005). While

the average rate of hospital infection in the USA and Europe is 10%, in Brazil it is around 15% (ANVISA 2004).

The control of hospital infections in Brazil has been regulated since 1982 by the Ministry of Health; however, only in 1997 was a Federal law (number 9431) formally established to compel hospitals to maintain a program of preventive and corrective actions related to the spreading of pathogens. Around 45,000 deaths are recorded yearly as a result of infections acquired in hospitals in Brazil, from a total of 12 million hospitalizations. General hospital infections are, unfortunately, the leading cause of deaths in intensive care units (ICUs) in Brazil, and waterborne-related contaminations play a significant role in this scenario. As a result of general nosocomial infections, the length of stay of a patient in a hospital can be extended by 10 to 14 days on average, generating costs of around \$5 billion annually in complementary treatments (APM 2006).

Organisms related to nosocomial infections are very diverse, being detected both in suspension and bound to surfaces in contact with water, in the form of biofilms. The source of microbial dissemination depends on many environmental factors, which can be minimized by specific control programs involving critical materials (surfaces, equipments and others) to prevent the occurrence of high contamination levels. Regular programs include periodical microbiological counts, visual inspection, and regular disinfection procedures, in accordance with the regulatory practice in force.

Wet surfaces and water storage and distribution systems are major sources of potentially pathogenic microorganisms that are not easy to detect or to control. The so-called waterborne pathogens include different types of bacteria, mycobacteria, fungi, parasites, and viruses (Anaissie *et al.* 2002a). Microbial biofilms, in particular, may be responsible for more than 65% of bacterial infections in the USA (Potera 1999), and this estimation was more recently increased to 80% by the National Institutes of Health of the same country (Lebeaux *et al.* 2013).

Microorganisms can easily adhere to piping systems and regions that accumulate water, especially those in which the water flow is difficult, forming biofilms. Relevant factors in the reduction of the overall quality of water and which favor the development of biofilms include the number and position of stagnant points in the water supply system, corrosion, and aging of the distribution system itself (pipe lines, connections, and storage tanks), as well as the formation of solid deposits on their surfaces. The contaminants may not only be transported by the running water system, but they may also be spread by the aerosol formed in taps and showers, dissipating easily in the environment. Surfaces conditioned by spills of contaminated water facilitate the deposition of other molecules and pathogens, and are prominent among the areas most favorable to microbial growth in hospitals.

As a result, the exposure of a patient to waterborne pathogens in a hospital may occur in many different situations, such as during a shower or a bath, while drinking water, due to the use of medical equipment rinsed with contaminated water, or to manipulation by medical personnel whose hands were previously washed with contaminated tap water (Shareef & Mimi 2008).

Biofilm-related infections are characterized by their chronicity and high resistance to antibiotics (Hanke *et al.* 2013), which makes microbiological diagnosis difficult and generally worsens a hospitalized patient's condition. In fact, in the worst scenario, the contact of a patient with waterborne pathogens can even lead to death, particularly in patients with compromised immune systems.

When in biofilms, microorganisms are more protected from the environment. Also, cells within biofilms interact more effectively through small secreted molecules (the quorum sensing concept), which enable them to better adapt to local chemical stimuli and to control the population density themselves due to the combination of intracellular signaling with modulation of gene expression (Camilli & Bassler 2006). Typically, Gram-positive bacteria secrete peptides, while Gram-negative bacteria secrete acyl homoserine lactones. As a result of population control, nutrient usage is better regulated and local permanence of the microbial community is more assured. In addition, many pathogenic bacteria are able to migrate from the environment to the human body and vice versa, having the ability to adapt to sudden responses of the host immune system, biofilm formation being a relevant example of microbial adaptation (Jefferson 2004).

HOSPITALIZATION AND OCCURRENCE OF INFECTIONS

For centuries, people who became ill were isolated in places with no natural light and no hygienic and dietetic care. Often, patients admitted for the treatment of an external injury or degenerative disorder died due to infectious diseases such as cholera, typhoid fever or suppuration. However, the development of new diseases and the death of those in isolation were associated with beliefs and superstitions. Over time, although extensive knowledge in microbiology was not yet a fact, the association between hospitalization and infection development was realized.

Conceptual and intellectual development, especially in the eighteenth century, made it possible for hospitals to perform more effective therapeutic actions, with questions raised about the conditions that favored microbial spreading, and by changing the design of hospitals from places where people were admitted to be excluded from social

life to institutions of healing and medication (Angerami & Andrade 1999).

Ignaz Philipp Semmelweis, a Hungarian obstetrician, is considered the forerunner in the control of hospital infections. In mid-1840, Semmelweis observed a difference in the number of cases of postpartum infections acquired in two clinics in a hospital in Vienna. In the first clinic, pregnant women were examined by doctors who were constantly present in the autopsy room, while in the second clinic, where the number of infections was substantially lower, the treatments were performed by midwives. On one occasion, one of the doctors was accidentally wounded by a knife while performing a necropsy, and developed an infection similar to that of the mothers. This fact led Semmelweis to conclude that the doctor had been contaminated by the same 'matter' affecting the patients, since at that time the concept of the existence of microorganisms was not well established. As a result, in 1847, Semmelweis made it compulsory for all employees of the hospital to wash their hands with a chlorine solution, thus drastically reducing the mortality associated with this problem from 12% to 1.9% (Veiga & Padoveze 2011).

RESISTANCE OF CONTAMINANTS TO ANTIMICROBIAL TREATMENTS

The use of systemic antimicrobial drugs on a large scale began in the 1940s, allowing treatment and the reduction of the number of cases of infections in hospitalized patients. However, military hospitals were soon confronted with *Streptococcus pyogenes* resistance to sulfonamide, a drug widely used at that time for the treatment of wounds. Similarly, the resistance of *Mycobacterium tuberculosis* to streptomycin occurred shortly after the introduction of this drug on the market. Disturbed by the infections in hospitals, the medical community received with enthusiasm other antimicrobial agents (Santos 2006), but soon after the initial use of penicillin hospitals were confronted with the resistance of *Staphylococcus aureus* to this drug. In the mid-1950s, outbreaks of resistant Staphylococcal infections were identified around the world, demonstrating the pandemic nature of the phenomenon. Later, in the 1960s, other microorganisms, especially Gram-negative bacteria

and fungi, were detected as agents of infections in hospitals (Santos 2006).

Interestingly, antimicrobial resistance was a driving force for health professionals and hospital administrators to recognize the need to establish procedures to monitor, control, and prevent the occurrence of infections developed during hospitalization. Such procedures have to take into account the main groups of occupants in a hospital, formed by patients, professionals, and visitors. These groups are different in terms of health status, exposure to infectious agents, susceptibility to developing diseases, and also regarding cross-transmission issues (Leung & Chan 2006), and all these factors demand great attention.

One of the main factors involved in the persistence of pathogens in the hospital environment is the improper use of sanitizers regarding type and concentration. This action may cause a false sense of disinfection, generating strains tolerant to different treatments performed in the water flow system, where the contaminants may then still proliferate. The same principle applies to the indiscriminate use of antibiotics, which favors subsequent microbial resistance to various treatments. Frequently, no direct relationship can be drawn between the effect of an antimicrobial agent on free cells and on cells organized in a biofilm, since besides the structural and physiological differences between both forms, the adherent cells in a given location may not be the same as those dispersed (Capelletti 2006). The concentration of an antimicrobial agent required to eliminate sessile cells (in biofilms) can be up to 1,000 times higher than that usually used on planktonic cells (in suspension) (Costerton *et al.* 1987; Capelletti 2006; Lucchesi *et al.* 2006).

COMMONLY FOUND WATERBORNE PATHOGENS

Ferranti *et al.* (2014), after compiling worldwide information from 125 scientific reports on waterborne healthcare-associated infections published in the period from 1990 to 2012, noticed that representative microorganisms of the families *Legionellaceae*, *Pseudomonadaceae*, *Burkholderiaceae*, *Mycobacteriaceae*, *Enterobacteriaceae*, *Moraxellaceae*, *Sphingomonadaceae*, *Xanthomonadaceae*, *Flavobacteriaceae*, *Aeromonadaceae*, *Campylobacteriaceae*, and Gram-

negative cells stand out as opportunistic environmental bacteria associated with this problem. A higher number of reports were determined for the families *Legionellaceae* (38.4% of the total), associated with pneumonia, *Pseudomonadaceae* (19.2%), frequently detected in respiratory tract and bloodstream infections, and *Burkholderiaceae* (12.8%), also related to bloodstream infections. The unit seen as the most commonly affected was the ICU, probably due to the frequently compromised physical and immunological condition of the patients. The primary source of Legionnaires' disease was shown to be the hot-water distribution system, while contamination of bottled water and of distilled and sterile water were mainly attributed to contamination by *Pseudomonaceae* and *Burkholderiaceae*, respectively. Most of the reports were from Europe (52.8%, of which 14 articles were from France and 11 from Germany) or from American countries (28.8%, of which 28 were from the USA). The occurrence of the problem in developing countries is certainly underreported.

ROLE OF WATER IN DISPERSION OF CONTAMINANT MICROORGANISMS BY AIR

Contact with microorganisms in normal environments is continuous but rarely noticed, unless it causes a disease or other deleterious effects. Indoors, air typically has about 1 million bacteria per cubic meter and tap water around 10 million bacteria per liter. Each microbial ecosystem has particular characteristics according to the environmental conditions of the place where it is installed (Feazel *et al.* 2009), and in hospitals, the occurrence of airborne contaminants' transmission is quite common. Assuming that the air of a given environment has a microbial concentration of around 1,000 colony-forming units (CFU) per cubic meter, and given that a person breathes normally 30 liters of air per minute, the load of inhaled microorganisms would be approximately 1,800 CFU every hour, while a conventional filter system with an average pore size of 0.5 micron processes about 90 CFU per hour (Lee *et al.* 2004). The viability of pathogens in the air is provided by water droplets or dust particles suspended in the environment for long periods. Microorganisms suspended in air may then be easily dispersed by air currents and be inhaled by a

susceptible host. Fungi of the genus *Aspergillus*, as an example, can affect approximately 15% of patients with leukemia and transplant, leading to death in around half of this population.

Generally, microorganisms in sessile form have strong virulence factors due to genetic changes that allow the synthesis of new protective substances that act outside and inside the cells. As reported by Kaur & Singh (2014), antifungal resistance is related to the capability of the extracellular matrix to adsorb antimicrobial agents, preventing their free diffusion to the contaminants inside the biofilm and also to the activation of multidrug resistance pumps during biofilm development, which may export biocide molecules from within the cells to the external environment. This combination of characteristics provides favorable cell survival conditions, which make cells in biofilms less susceptible to elimination when compared to the same microorganisms in planktonic form (Morck *et al.* 2001).

Despite the implementation of prophylactic procedures for the control of airborne contaminants in a given location, the water distribution system frequently acts also as a reservoir of opportunistic microorganisms. Sections of piping where water tends to stagnate provide good growth conditions for pathogens. The concentration of microorganisms dispersed in air increases in areas with intense use of water, which strengthens the transmission of pathogens to the environment (Anaissie *et al.* 2002b). The level of humidity of the surfaces near points of water use can be an important indicator in helping prevent the establishment of contaminants. Even moisture levels as low as a little above 20% may facilitate the development of microorganisms and their dissemination on absorbent structural items such as carpeting, wallboards, and wallpapers if these materials are not properly dried within 72 hours after wetting (Centers for Disease Control and Prevention 2003).

Under adequate growth conditions, a bacterium with a doubling time of around 20 minutes can generate more than two million cells in 8 hours. Given that small amounts of substrate can fulfill the nutritional needs of the contaminants and that concentrations as low as one part per billion of organic matter in 1 milliliter of water may make possible the growth of approximately 9,500 bacteria (Dreeszen 2003), it is clear that water systems have the potential

not only to disseminate contaminants but also to support their propagation.

CRITICAL AREAS IN HEALTHCARE FACILITIES REGARDING MICROBIAL DISPERSION THROUGH WATER

Nosocomial infections originating from water can be transmitted not only by aspiration, but also by contact and ingestion. Many pathogens can survive in hospital water supply systems, transferring antibiotic resistance genes and being implicated in numerous outbreaks.

Among the highest water consumption areas in a hospital are steam generators, hemodialysis equipment, laboratories, surgical materials processing sections, air conditioning systems, and laundries (Anaisie *et al.* 2002b). The main reservoirs of pathogens in clinical settings reported in the literature are drinking water, water for dialysis, water used for washing medical devices, water used in taps and showers, water lines in dental clinics, and eye washers (Centers for Disease Control and Prevention 2003).

Proper guidelines for the monitoring and prevention of hospital waterborne infections are still limited. In recent years, increases in the occurrence of pathogenic fungi and molds in hospital areas have been detected (Falvey & Streifel 2007), and studies pointing to contaminated surfaces and water supplies as possible sources for aspergillosis (Streifel *et al.* 1987; Anaisie *et al.* 2002c) thus raise the need to formulate general and specific guidelines for monitoring hospital water sources. Avoidance of drinking hospital tap water, routine and targeted surveillance cultures for water sources, and hospital staff and patients' education are major measures to control water-associated nosocomial infections.

Monitoring and detection of the transference of pathogens from water to medical instruments are not frequently performed and can lead to incorrect diagnosis of infection. Data provided by a study of nosocomial infections related to water sources (Pall Corporation 2006) showed that devices commonly involved in microbial transmission include not only taps but also nebulizers, affecting patients with respiratory problems, and burns, neonates, patients recovering from cardiac surgery and neurosurgery, as well as the elderly, who are particularly vulnerable. According

to the instructions of the Centers for Disease Control and Prevention (2003), for cleaning medical materials, such as endoscopes and bronchoscopes, the water must be of high quality to avoid microbial growth and biofilm formation within these devices.

A study of disinfection in an Italian hospital contaminated with *Legionella pneumophila* was performed by circulating peracetic acid through the piping system (Ditommaso *et al.* 2005). *In vitro* tests showed that the effective concentration for contaminant inactivation in the system was 50 ppm after 5 minutes of contact. Based on these results, a four-step disinfection protocol was then established. In the first step, the disinfectant was used at this dose but for a contact time of 30 minutes. In the second step, the treatment was repeated weekly for 3 weeks, and in the third step, the disinfection was performed in the same conditions of dosage and contact time, and repeated every month for 5 months. Finally, in the last step, the dosage was raised to 1,000 ppm of peracetic acid for a 30 minute exposure period. Despite the multiple disinfection steps, the growth of the same bacteria was detected again 30 days after the procedures, in a concentration even higher than the initial one, due to remaining cells in the form of biofilms within the water pipes, which protected the microorganisms from the disinfecting agent (Ditommaso *et al.* 2005).

Shower use can provide a source of exposure to microorganisms through aerosolization, as the inside of a showerhead provides a moist, warm, and dark environment that is frequently replenished with nutrients. The heating provided by shower water systems is obviously not hot enough to overcome the transmission of microorganisms, and most of the microbiota found in these devices is composed of groups commonly found in water and soil capable of forming biofilms in favorable conditions (Feazel *et al.* 2009). A shower system may include a reservoir of bacteria such as *Legionella*. As a result of the warming of the water in showers, this microorganism may easily spread and reach the respiratory system of the patient. In addition, water drains often cause problems in hospitals if overflow occurs, spreading pathogens on the floor surface (Prade *et al.* 1995).

Showers and taps in hospitals may also be a significant source of fungi that cause infections in patients with

weakened immune systems. In 2001, a detailed study was performed focusing on the route of transmission of *Aspergillus* related to hospital showers and taps (Warris *et al.* 2001). In this study, a total of 100 samples of this fungus were collected from air, water, and patients in a hospital in Norway. Among the samples analyzed, 55 were collected from the water system (51% in taps, 44% in the main piping system, and 5% in showers), 25 were obtained from the air, and 20 originated from 13 immunocompromised patients. The samples collected from the water were genetically distinct from those obtained from the air. However, in nine of the 13 patients evaluated, *Aspergillus* strains genetically similar to those found in the water system were detected.

Although opportunistic pathogens have been cultured from showerheads, little is known about either the prevalence or the nature of the microorganisms that can be aerosolized during showering. To determine the composition of showerhead biofilms and water, in 2009 a study was carried out focusing on the ribosomal RNA gene sequences of biofilms from 45 showerheads from nine sites in the USA (Feazel *et al.* 2009). The authors found that sequences representative of non-tuberculous mycobacteria and other opportunistic pathogens were highly frequent in many showerhead biofilms.

The development of cyanobacteria (blue algae) in drinking water reservoirs, which culminated in a toxic syndrome known as toxic pneumonia, was reported in Scandinavia (Annadotter *et al.* 2005). Symptoms such as fever and signs of respiratory tract failure were usually detected in only 1.5 to 6 hours after people had bathed, and the presence of endotoxins dispersed in the aerosols generated during the bath were reported as the probable causative agent.

In Brazil, in 1996, a major outbreak of waterborne nosocomial infection occurred in the town of Caruaru, Pernambuco, affecting 131 patients with chronic renal failure undergoing hemodialysis. Of these, 46 died due to intoxication by microcystin produced by the algae present in the water circuit (FAPESP 1996).

As already mentioned, several pathogens can affect debilitated patients, such as *Escherichia coli*, *Klebsiella*, *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Nocardia* spp., *Mycobacterium* spp., *Haemophilus influenzae*, and *Neisseria meningitidis* (Nucci & Maiolino 2000), among others. However, some microorganisms are

noteworthy, both in number and type of infection, such as the bacterium *Pseudomonas aeruginosa*, which is often found in different regions of the body and the environment due to being easily adaptable to different conditions. This bacterium is ubiquitous in water and has been responsible for mortality rates around 30% among patients with pneumonia and sepsis and 60% in burned patients (Angelbeck 2004). Bacteria such as *L. pneumophila* can cause pneumonia during hospitalization, both through contaminated water and by airborne transmission, while *Serratia marcescens* may usually be associated with pneumonia and sepsis in patients undergoing chemotherapy. The last mentioned microorganism is slow growing, has invasive properties and the tendency to resist many of the antibiotics used nowadays (Koneman *et al.* 2001). Additionally, *Methylobacteria*, a group characterized by being composed of slow-growing microorganisms resistant to chlorine-based treatments, are described as important pathogens transmitted by water (Hiraiishi *et al.* 1995). Also noteworthy are the *Mycobacteria*, which are capable of survival at extreme temperatures, such as in ice machines and hot water, particularly the *intracellulare* species, which can persist for more than a year in distilled water. Other bacteria commonly found in drinking water and of great importance regarding the incidence of infections include *Stenotrophomonas maltophilia*, *Aeromonas hydrophila*, *Acinetobacter* spp., *Enterobacter* spp., *Flavobacterium* spp., and *Burkholderia cepacia* (Angelbeck *et al.* 2006).

Another microorganism associated with major concern is *Acinetobacter*, due to its rapid ability to develop resistance to many antimicrobial agents, including several antibiotics and heavy metals (Akbulut *et al.* 2014). This bacteria may demonstrate hemolytic activity, and if infecting a hospitalized person, its discharge through untreated or only partially treated hospital contaminated wastewater may direct it to surface waters, where it is capable of persisting for extended periods, continuing the contamination and spreading cycle.

Fungi are responsible for approximately 8% of total hospital infections, the ones from the genus *Aspergillus* being the most important regarding infections in immunocompromised patients, particularly those from the species *flavus*, *niger*, and *fumigatus* (ANVISA 2004), which may cause death in about half of the patients affected.

Therefore, taking into account the high prevalence of contaminants in water, various contamination control alternatives complementary to the use of chemical agents are being considered in many hospitals, particularly in Europe. In Germany, since over 40% of infections by *Pseudomonas* spp. in intensive care units were associated with the use of water, a growing number of disposable filters have been installed in taps and showers for the protection of patients (Reiter 2004). The installation of these filters is considered a very cost-effective alternative if elements such as the expense of rehabilitation of the affected patients and measures to treat the contaminated area are taken into account. It was observed that the installation of only seven filters in a hospital reduced substantially the infections, with savings of approximately 82% in the total usually spent to circumvent the problem.

In severe cases, more aggressive intervention strategies are required and sometimes the only possible measure to prevent or stop the process of infection in patients with high risk is restriction of water use (Squier *et al.* 2000) and establishing very strict water quality control standards. As an example, healthcare guidelines of the Centers for Disease Control and Prevention (2003) state that for dialysis water, microbial count levels below 200 CFU per milliliter are recommended.

The severity of the problem is illustrated by a case related to the intensive care unit of a hospital in France, in which the bacterium *P. aeruginosa* was detected in approximately 10% of 657 samples of tap water collected (Rogues *et al.* 2007). The percentage of transmission of this contaminant particularly through the hands of the local health workers was 14%, with the same strain being isolated from 38 patients. This case report strengthens the concept that among the many sources responsible for nosocomial infections, hospital water is a controllable but surely overlooked one.

SYSTEMATIC MONITORING AND CONTROL OF HOSPITAL INFECTIONS: OVERVIEW IN BRAZIL

Although in Brazil the first hospital infection control committees arose in the 1960s (Padoveze & Fortaleza 2014), the Brazilian National Agency of Sanitary Surveillance (Agência Nacional de Vigilância Sanitária, ANVISA) was

only officially instituted in 1999. Since then, this agency has been responsible for the national program of prevention and control of infections related to healthcare facilities. However, to our knowledge, no systematic and detailed studies on nationwide statistics of the occurrence of hospital infections exclusively related to waterborne microorganisms and biofilms are available in the literature.

An analysis of the magnitude of general nosocomial infections in Brazil was performed by the Department of Infection Control in Hospitals of the Ministry of Health, involving 99 hospitals located in different capitals of Brazilian states, totaling 8,624 patients (Prade *et al.* 1995). The average hospital stay of patients affected by nosocomial infections was 21.7 days and the infection rate was 13%. Prevalence was observed for respiratory tract (28%), followed by surgical (15%), skin (15%), and urinary-related (11%) cases. In a situation different from what is now seen, it was noticed in 1995 that 46% of the patients in surgical clinics and 24% of patients in regular clinics used antibiotics without apparent infection or diagnostic, a practice that favors the development of microbial resistance and complications of further treatment. The southeast region had at that time the highest prevalence of nosocomial infections (16.4%, 37 hospitals), followed by the northeast (13.1%, 27 hospitals), north (11.5%, eight hospitals), south (9.0%, 15 hospitals), and midwest (7.2%, 12 hospitals). The nature of the hospitals was taken into consideration, and public hospitals that had higher rates of infection (18.4%) were compared to teaching hospitals (11.8%) and to those of the private sector (10%).

In 2007, a nationwide search was performed to analyze the existence of committees for hospital infection control, as well as for microbiological laboratories in Brazilian hospitals (ANVISA 2013). According to the reported information, only 4.3% of the evaluated institutions had the support of municipal committees for the control of hospital infections. Moreover, it was detected that in approximately 40% of the hospitals, microbiology laboratories were unavailable. This hampers the adoption of policies for the rational use of antimicrobial agents and also contributes to increase the risk of treatment failure in patients with infectious diseases. It was noted that effective measures for monitoring, evaluating, and reporting of nosocomial infection indicators needed to be improved. This

study also shows that hand washing was identified as one of the most relevant items related to infection control, as also stressed by *Borges et al. (2012)*; nevertheless, water itself is seldom recognized as a potential source of contaminants. Obviously, despite hand washing being a simple, inexpensive, and effective measure to prevent the spreading of pathogens in the hospital environment (*El-Far & Richtmann 2001*), the water used to do it must have adequate microbiological quality.

An investigation performed from 2007 to 2008 in a state hospital of Sumaré, in São Paulo State, showed that from the 862 deaths observed in that period, around 9% were associated with nosocomial infections (*Guimarães et al. 2011*). Although bacterial resistance was not the focus of that particular study, multidrug resistance rates above 30% for Gram-positive cells and over 40% for Gram-negative cells were detected.

Similarly, according to the National Agency of Sanitary Surveillance in Brazil, in 2007, 64 hospitals reported multidrug resistance of cultures of the bacterium *P. aeruginosa*, commonly found in water (*ANVISA 2008*). On average, only 58% of the tested cultures showed susceptibility to at least one of nine major antimicrobials used in conventional antibiotic therapy (amikacin, gentamicin, levofloxacin, ciprofloxacin, meropenem, imipenem, cefepime, ceftazidime, and tazobactam).

In 2013, a survey on the prevalence of healthcare-associated infections in Brazilian hospitals was carried out, in which 91 hospitals were evaluated (*Fortaleza et al. 2013*). The overall infection rate was 11.1%, varying from 2.5% (hospitals with less than 50 beds) to 18.3% (hospitals with more than 200 beds). The most prevalent infections were pneumonia (3.6%), bloodstream infection (3.5%), surgical site infection (1.4%), urinary tract infection (1.1%), and skin infection (0.4%). The risk factors more frequently identified were: central venous catheter (17.8%), surgery (15.5%), urinary catheter (14.0%), and mechanical ventilators (8.1%). Etiologic agents were identified only in 9.1% (43 of 473) of infections. Gram-negative organisms were more frequent (56.0%) and, among them, *Klebsiella* spp. (19.0%) and *P. aeruginosa* (16%) were predominant. Among Gram-positives (35.0%), coagulase-negative *Staphylococci* were more prevalent (16%) than *S. aureus* (9.0%) or *Enterococcus* spp. (6%). Yeasts were identified in 9.0% of the infections in this study, and in a former survey, molds

were also found to be relevant as hospital waterborne contaminants in Brazil (*Varo et al. 2007*). The monitoring of seven points of distribution of water in a hemodialysis unit in the state of São Paulo, from April to July 2006, indicated the presence of 116 isolates of filamentous fungi, of which 41% were *Trichoderma* spp., 25% *Cladosporium* spp., 14% *Aspergillus* spp., and 10% *Fusarium*.

A recent analysis (*ANVISA 2013*) showed that among the elements recommended for evaluation by the World Health Organization (*WHO 2011*), the items that better met the international compliance standards with regard to prevention and control of nosocomial infections in Brazilian healthcare institutions are vigilance, technical guides, and environment. Monitoring, evaluation, and relation to public health, however, did not reach adequate levels.

Owing to being among the 10 largest economies in the world, Brazil's situation regarding statistics, prevention and control of infections related to healthcare facilities attributed to waterborne microorganisms and biofilms may be potentially correlated to that of other developing countries in the BRICS group (Brazil, Russia, India, China and South Africa, which represents more than 40% of the world's population) and also of other nations. Therefore, the data presented herein could well serve to instigate more thorough assessment of the problem and also of ways to more effectively deal with it.

CONTAMINATION CONTROL IN WATER SYSTEMS: TRADITIONAL METHODS AND INNOVATIONS

In natural environments, microorganisms are mostly found as biofilms, in sessile communities, consisting of microbial associations of interdependent species that can colonize and develop on various types of surfaces. The cells in biofilms are protected by an extracellular polymeric matrix (EPS) of complex and heterogeneous composition, which promotes microbial attachment, proliferation, and differentiation. Owing to displaying hydrophilic and hydrophobic regions, the EPS enables the development of biofilms on different materials (*Tsuneda et al. 2003*).

Biofilms are formed in a sequence of events, which may vary according to the microbial flora present and cell adaptation to different media (*Figure 1*). Initially, planktonic cells

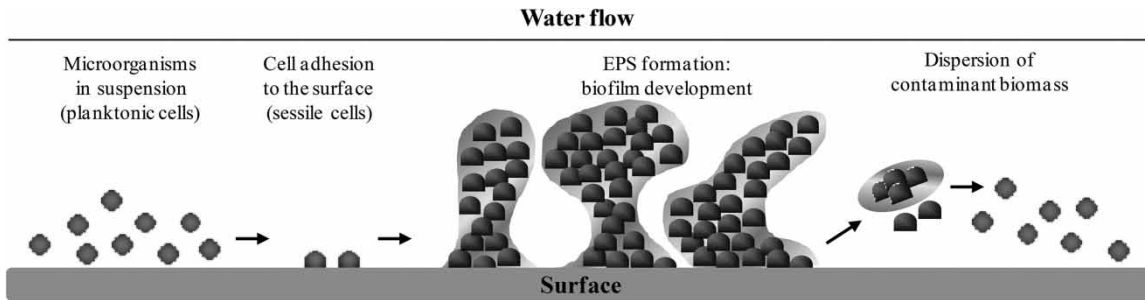


Figure 1 | Major steps involved in biofilm formation. Planktonic cells attach to the surface, forming an extracellular polymeric matrix that stabilizes the biofilm and through which the fluid is able to circulate. Extracellular and intracellular signaling activates modulation of gene expression, contributing to improved cell resistance and survival. Upon biofilm maturation, daughter cells and microcolonies may be released and dispersed in the fluid stream, thus being able to colonize other surfaces.

are transported to the surface of the liquid phase by sedimentation, diffusion, or convection. Then, cell adhesion to the surface occurs, normally through weak forces in an initially reversible step, and afterwards through less reversible forces such as ionic or covalent bonds. After adhesion, cell growth effectively takes place and the structure of the biofilm stabilizes as a whole with the formation of the EPS, through which circulates the fluid in the vicinity of the biofilm. The biofilm is then considered mature, releasing cells as a result of quorum sensing or nutrient level control, biofilm tearing due to continuous liquid flow or by shedding of daughter cells. The released cells may, in turn, colonize other surfaces, restarting the contamination cycle.

Currently, the most used methods to prevent and control microbiological contamination on surfaces can be divided basically into three categories: mechanical cleaning procedures, use of sanitizing agents, and use of antimicrobial coatings or membranes. However, there are many factors that may contribute to microbiological contamination of water and culminate in biofilm formation. The misuse of disinfection methods is among the most problematic, because in this way the elimination of the contaminant focus may not be obtained. Some of the most frequently used approaches to disinfect water are compiled in [Table 1](#). Nonetheless, their limitations should be considered when selecting a specific treatment ([Schindler 2001](#)).

Several strategies can be employed to control the infection rates originating from water in hospitals ([Curtis 2008](#)), including simple measures such as using sterile water as drinking water and in showers. Also, cleaning of showers with detergents and phenolic compounds, heating water at temperatures above 50 °C, and immediate repair of leaks

and damages resulting from water flow are rather effective. More elaborate strategies are also available, such as water treatment with UV light or ionization systems based on copper and silver. The use of chemical agents is also indicated, however most of them, even at dosages above the usual, are unable to completely and permanently eradicate biofilms already installed, which develop again and may turn resistant ([Angelbeck *et al.* 2006](#)).

In general, the concentration of chlorine necessary to eradicate most of the microorganisms present in water is approximately 0.3 milligrams per liter. However, even with the addition of free chlorine in water pipes at concentrations as high as 4.3 milligrams per liter, some coliforms can survive ([LeChevallier *et al.* 1984](#)). This can be attributed to some common factors in water circuits: the chlorine added may not reach all areas of the water distribution network in sufficient quantity for its action, and part of the chlorine added may react with traces of pre-existing organic matter or corrosion products, among other possibilities. Even portions of biofilms detached from surfaces as a result of the action of disinfectants can be problematic. Such fragments can serve as a source of easily assimilable organic carbon for the maintenance of the living microorganisms remaining in the system.

In most cases, the cost for the treatment of biofilm-related contamination is much greater than the amount that would be spent if there were actions to prevent its occurrence. As mentioned previously, a current alternative, very attractive and with proven efficacy, is the use of filters at points of final consumption, such as in taps and showers ([Ortolano *et al.* 2005](#); [Sheffer *et al.* 2005](#); [Exner *et al.* 2005](#); [Lin *et al.* 2011](#)). Point-of-use water filtration is one of the particular strategies recommended by [Lin *et al.* \(2011\)](#) for

Table 1 | Relevant strategies of water disinfection and their characteristics (compiled from Schindler 2001; EPA 2011; Lin *et al.* 2011)

| Disinfection technique | Advantages | Disadvantages |
|----------------------------|--|--|
| Flow of hot water | Does not require specialized equipment Does not involve the use of chemical agents | Risk of burns Damage to pipes Difficulty in reaching the whole area in complex distribution systems |
| Chlorination | Good short-term efficacy Well understood disinfectant capability Established dosing technology | Requires periodic analysis of the chlorine level <i>Mycobacteria</i> and <i>Legionella</i> are potentially resistant Ineffective against <i>Cryptosporidium</i> Development of odor, allergic reactions, and carcinogenic byproducts (trihalomethanes) Corrosive Does not permeate effectively in biofilms |
| Ionization (copper/silver) | Good efficacy in short- and long-term use Easy equipment installation and maintenance Accumulation of ions inside the biofilm considered as the basis for the prolonged bactericidal effect | Water must present low concentration of dissolved solids High water pH and low ion concentrations may affect the method's efficacy Requires routine maintenance and monitoring (every week for copper and once every 2 months for silver) Only effective with flow of hot water Corrosive to steel and galvanized pipes |
| Exposure to UV light | Easy installation Pronounced action in planktonic cells Does not require the use of chemical agents No significant by-product implications Generally highly effective for protozoa, bacteria, and most viruses and particularly for <i>Cryptosporidium</i> | Poor penetration in biofilms Frequent microbial recolonization Water supply should not be turbid for higher treatment efficacy Difficulty in reaching the whole area in complex distribution systems Efficacy is reduced by high water flow, presence of organic materials, and high microbial levels High costs No residual effect distributed to the remainder of the system |
| Ozonization | Good short-term efficacy Benefits of destruction of organic micropollutants (pesticides, taste and odour compounds) Strong oxidant and highly effective disinfectant compared with chlorine | Requires specialized equipment which is difficult to install and maintain High costs Action limited to the injection point Fast decomposition of ozone Questionable effect on biofilms Residual effect insufficiently long lasting for distribution over the system under treatment |
| Chloramination | No significant by-product issues | Considerably less effective compared with chlorine |

(continued)

Table 1 | continued

| Disinfection technique | Advantages | Disadvantages |
|------------------------|---|--|
| | Generally less taste and odour issues than chlorine | Monochloramine can cause anemia in patients undergoing hemodialysis |
| | Stable monochloramine residual penetrates biofilms | Increased populations of other microorganisms (<i>Mycobacterium</i> species) |
| | Wider working pH range than copper/silver ionization and chlorine | Presence of nitrogen by-products and increased lead leaching in drinking water |
| | | Use of monochloramine generally limited to municipal water treatment plants |

emergency disinfection methods in the case of hospital-acquired Legionnaires' disease, in addition to the use of superheat-and-flush disinfection and/or shock chlorination.

The high efficiency of the approach based on installing point-of-use water filters was recently reported by [Zhou et al. \(2014\)](#). The filters were capable of eliminating *Legionella* spp., *P. aeruginosa*, *Mycobacterium* spp., and filamentous fungi from the tap water of a liver transplant unit in a hospital in Shanghai, China, also reducing the incidence of colonization and infection with Gram-negative bacteria by 47%.

Another example of the successful use of the filtration strategy is described by [Vianelli et al. \(2006\)](#), who reported the use of disposable filters with 0.2 µm pore size at points of consumption such as taps and showers in bathrooms at hematology and oncology areas in an Italian hospital. Such an approach not only allowed a significant reduction of *P. aeruginosa* bacteremia, but also contributed to the control of infection outbreaks involving the same organism. The authors also point out that despite the increase in the annual operating costs due to changing the filters weekly, a significant contribution to the reduction of morbidity, consumption of antibiotics, and length of stay of patients in the hospital was noticed.

The filtering approach can be used as a complementary procedure to chemical disinfection treatments, with the advantage of capturing microorganisms that may have survived exposure to these agents or have not been reached in stagnant regions of the piping system.

A comparative study of different strategies to control *Legionella* spp. in a hot water supply, conducted at a university hospital in Italy for 10 years ([Marchesi et al. 2011](#)),

showed that filters placed directly in water use points perform best with respect to the reduction of contamination, followed by the use of heating, chlorine dioxide, heat shock, and hyperchlorination. The use of chlorine dioxide, however, is the least expensive procedure followed by thermal shock, hyperchlorination, heating, and filtration.

Although cost is a relevant factor in the analysis, strategies for high efficacy in microbial control of water and based on a combination of two or more distinct principles of disinfection can be vitally important in sectors where hospital treatments are carried out on severely immunocompromised patients. Strategies also comprehending the use of devices and materials of extremely low risk to patients and to the environment, such as those based on the use of natural-origin bioactive compounds like chitosan, are being increasingly considered, mostly to coat surfaces prone to short-time contact with moisture.

ALTERNATIVE APPROACHES TO PREVENT WATERBORNE NOSOCOMIAL INFECTIONS USING THE BIOPOLYMER CHITOSAN

Chitosan, a polymer obtained by deacetylation of chitin, a polysaccharide that has a structure similar to cellulose, has attracted great interest for application in the biomedical area lately due to its antimicrobial properties (as a biocide and biostatic agent) ([Chandy & Sharma 1990](#)). Its use as a natural coagulant for the treatment of drinking water in the isolated form or together with other approaches is also well documented ([Lee et al. 1992](#); [Eikebrokk & Saltnes 2001](#); [Fabris et al. 2010](#); [Khaira et al. 2013](#)).

Besides these attributes, chitosan is a versatile material that can be used alone or in combination with other compounds, aiming at improving its physical, mechanical, and/or biological characteristics for specific applications. It can be processed in different forms, such as solutions, gels, particles, dense and porous films and membranes, among others, and has low toxicity to humans. As a consequence of all these attractive characteristics, added to its high availability, its use in the development of biomaterials has been increasingly investigated in recent years, with great emphasis on the production of wound dressings (Jaya-kumar *et al.* 2011).

Chitosan has the capacity to inhibit the growth of a wide variety of bacteria, molds, and yeasts (Singla & Chawla 2001; Raafat & Sahl 2009). However, the presentation form of the final material can significantly influence its antimicrobial activity (Foster & Butt 2011). The high density of positive charges in chitosan molecules is highlighted in several studies as one of the main factors involved in its mode of action, propitiating the interaction with microbial cells and their toxins, which are typically negatively charged. The cell wall composition of many organisms commonly found in water, such as cyanobacteria, is similar to that of Gram-negative bacteria, which also have negative charges in their surface (Cossich 2000). The reproductive structures of some filamentous fungi are also negatively charged (Dunlap *et al.* 2005), as well as the surface of common yeasts (*Saccharomyces* spp. and *Candida* spp.), which in all of the situations described would favor the interaction of cells with chitosan. In this sense, chitosan is successfully used as a flocculating agent to remove impurities in chemical and biological water treatment (Strand *et al.* 2002).

Studies involving the use of chitosan as a coating for surfaces indicate that this method of antimicrobial protection provides a promising field of application in the control of nosocomial pathogens (Wang *et al.* 2012; Cobrado *et al.* 2013). However, the intrinsic bactericidal activity of chitosan seems to be more intense in preparations in the form of solutions or gels than in neutralized materials (Foster & Butt 2011). It is assumed that there is a significant contribution to the chitosan antimicrobial effect from the organic acids commonly used to solubilize this polysaccharide due to the pH reduction of its solutions or gels (Chung *et al.* 2003; Fujimoto *et al.* 2006). Consequently, the antimicrobial

activity observed for chitosan and its derivatives is perceptible only when the pH is below the dissociation constant of the amino groups of the respective compounds. This mechanism is not limited to soluble forms of chitosan, but is also verified in solid chitosan samples (Kong *et al.* 2010). Thus, when the use of neutralized chitosan films at basic or neutral pH conditions is desired, the chitosan device should ideally be combined with compounds having microbicidal activity to more effectively control the development of microbial biofilms.

Styrene-acrylic coupons coated with this polymer and exposed to clinically relevant microorganisms such as *Staphylococcus epidermidis* and *Candida albicans* showed enhanced antifouling activity in comparison to coupons treated with conventional antimicrobial agents (Carlson *et al.* 2008). In the same type of application, chitosan in the form of a neutralized film in combination with the antibiotic rifampin has already been successfully used for controlling the development of *S. epidermidis* and *S. aureus* biofilms (Cao & Sun 2009).

Other prospects for application of this biopolymer in microbial control of water used in hospitals should be further explored, both directly as a potential antimicrobial agent in solution and in an indirect way as a matrix for the incorporation of other antimicrobial agents.

CONCLUSION

The number of cases of infections of nosocomial origin associated with systems of water distribution in hospitals around the world is highly significant. The development and adoption of more effective measures to prevent its progression is an assured need, as is providing qualified information on this matter to professionals working in healthcare facilities and also to patients and their companions, mostly in developing countries, where activities on prevention, monitoring, and control of waterborne contaminants tend to be more limited. It is essential that when the use of antimicrobial agents cannot be avoided to overcome waterborne pathogens' replication and spreading, these compounds should be employed in a rational way to minimize the major problem of development of microbial resistance to their presence. Despite the fact that filtration

systems are particularly cost-effective as alternative or complementary approaches to control waterborne contaminants in hospitals, the use of antimicrobial agents of natural origin, such as chitosan, should be more frequently considered for the purpose of reducing the risk of nosocomial infections together with other useful strategies.

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