Chapter

Sanitation and the Environment

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Abstract

The environment is severything that creates natural conditions for the existence of organisms, including humans, and is a prerequisite for its further development. Proper environmental hygiene can prevent the outbreak and spread of infectious diseases. The function of disinfectants is to kill and prevent the growth of microorganisms. Disinfectants are potentially noxious substances which are used in intensive animal production and disease control programmes. In fulfilling this role, disinfectants may also have an adverse impact on the environment. These products may harm beneficial microorganisms, plant and animal life, and even humans, when used without due caution. Proper selection of disinfectant which is based on the knowledge of the resistance of microorganisms to the effect of the disinfectant and the efficacy of the disinfectants as well as the potential negative impact on the environment minimizes the risk of microbiological contamination and improves quality of the environment.

Keywords: sanitation, microorganisms, environment, disinfection, hygiene, disinfectants

1. Introduction

One of the most significant environmental problems of the present, affecting all environmental components, is global environmental contamination, which is closely linked to the unprecedented boom in industrial and agricultural chemistry. The environment, both natural and artificial, is one of the factors that affect human health and well-being. The relationship between the environment and health, the so-called environmental health, should be understood as a complex of interactions between the genetic characteristics of a human being and the environment in which she lives. Exposure of men to environmental pollutants can trigger the onset of diseases, most often chronic [1]. Environmental contamination plays an important role in the transmission of several key health care-associated pathogens. Effective and thorough cleaning/disinfecting of the patient environment is essential. Although microbiologically contaminated surfaces can serve as reservoirs of potential pathogens, these surfaces generally are not directly associated with transmission of infections to either staff or patients. The transferral of microorganisms from environmental surfaces to patients is largely via hand contact with the surface. Although hand hygiene is important to minimize the impact of this transfer, cleaning and disinfecting environmental surfaces as appropriate is fundamental in reducing their potential contribution to the incidence of healthcare-associated infections.

The principles of cleaning and disinfecting environmental surfaces take into account the intended use of the surface or item in patient care [2]. Protecting human, animal and plant healthiness at every stage of the food production process is one of the top priorities for the public health and economy. Food safety is becoming increasingly of interest to consumers and producers, and microbiological purity of food raw materials, technological equipment, production areas and final products is inseparably linked to it. Therefore, a great emphasis is placed on the whole quality assurance complex, including production hygiene [3]. Foodborne diseases encompass a wide spectrum of illnesses and are a growing public health problem worldwide. They are the result of ingestion of foodstuffs contaminated with microorganisms or chemicals. The contamination of food may occur at any stage in the process from food production to consumption ("farm to fork") and can result from environmental contamination, including pollution of water, soil or air [4]. Environmental sanitation is the promotion of hygiene and the prevention of disease and other consequences of ill-health, relating to environmental factors. To allow for transmission of infectious agents they have to be present in the immediate human environment, exposure has to take place, and transmission has to occur by uptake of the agents through unsafe practices. To interrupt the transmission, environmental sanitation can act on reducing exposure to infectious agents by limiting contact to wastes or polluted media, and by changing hygiene and socio-cultural practices. Sanitation is the effective use of tools and actions that keep our environment healthy. Sanitation is a complex of measures directed to the inactivation, removal, or killing of the agents of infections in the external environment. Sanitation includes disinfection, insect control, rodent control, proper disposal of wastes (cadavers, excrements, wastewater) and hygiene of the environment [5].

2. Disinfection

Disinfection is defined as a process in which germs are destroyed by either chemical action or physical intervention, or a combination of both [6].

2.1 Stages of disinfection

Disinfection has several stages. The first stage is the **exploratory and preparatory work** to determine the extent and type of disinfected object. The necessary amount of tools, aids, appliances, employees and effective disinfectant must be provided [7]. Cleaning, second stage, is the necessary first step of any disinfection process. Cleaning removes organic matter, salts, and visible soils, all of which interfere with microbial inactivation. The physical action of scrubbing with detergents and surfactants and rinsing with water removes substantial numbers of microorganisms. If a surface is not cleaned first, the success of the disinfection process can be compromised. Removal of all visible blood and inorganic and organic matter can be as critical as the germicidal activity of the disinfecting agent. When a surface cannot be cleaned adequately, it should be protected with barriers. It has been estimated that cleaning alone may remove over 90% of bacteria from surfaces [8]. The third stage is the **actual disinfection**, its objective being to destroy the decisive number of microorganisms that remained on the objects and surfaces after mechanical cleaning. The fourth stage is **to check the effectiveness of disinfection**. The effectiveness of surface disinfection must be controlled. The inspection informs about the quality of the work done and about the effectiveness of the disinfectants used. In the case of identified insufficiencies, it is the basis for the implementation of corrective measures. The effectiveness of disinfection is checked by chemical and microbiological swabs. Chemical methods are divided into qualitative and quantitative. The methods are very costly and time consuming, thus they are rarely done. Methods are currently available to detect undesirable residues of disinfectants on surfaces as well as in products. Microbiological swabs can be used to verify that microorganisms have been killed on disinfected areas and objects [9]. The fifth stage is **ventilation and deactivation**. This stage is only performed after the disinfectant exposure time necessary for action has expired. Residues of disinfectants on surfaces and objects are removed through rinsing with water; in some cases, inactivating substances are used. A protocol shall be drawn up after each disinfection carried out [10].

2.2 Environmental factors influencing the effectiveness of disinfection

The effectiveness of disinfectants for microorganisms depends on many factors. On the one hand, these are the properties of the microorganisms themselves, on the other hand, the chemical and physical properties of the external environment. Knowing these factors should lead to a more adequate use of disinfection. The number and types of microorganisms present on environmental surfaces are influenced by the following factors:

a. number of people in the environment

b. amount of activity

c. amount of moisture

d. presence of material capable of supporting microbial growth

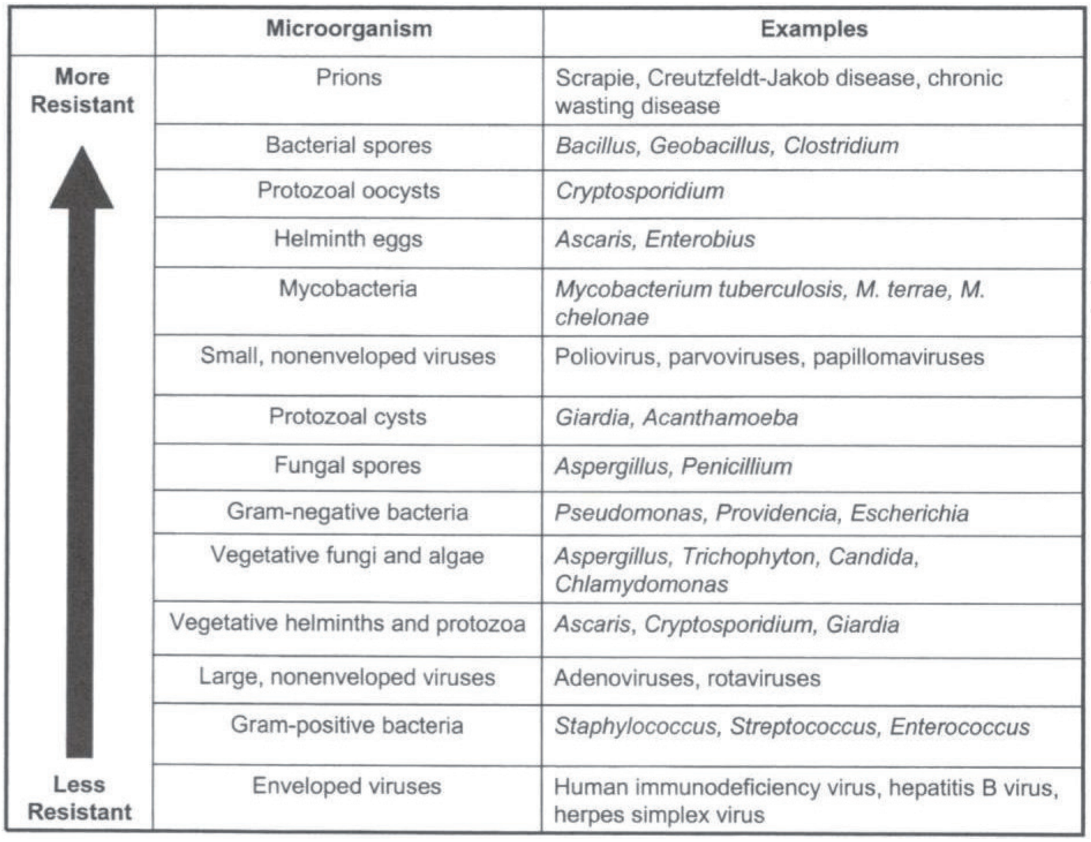
e. rate at which organisms suspended in the air are removed

f. type of surface and orientation (e.g. horizontal or vertical) [10].

Factors that affect disinfection efficiency include:

- the concentration of the disinfectant
- the time during which the microorganism is in contact with the disinfectant
- pH
- temperature
- the presence of organic contaminants, e.g., blood, serum or other body fluids
- the microorganism itself or the respective agent, their type (prions, viruses, gram-negative, gram-positive bacteria, microscopic fungi, protozoa, spores) as well as their number and location [11].

The most important factor is the concentration of the disinfectant from which the effectiveness of the disinfection depends. The mechanism of action depends on the chemical composition of the disinfectant and the way it is used. In the case of unstable disinfectants (chlorinated lime, formalin, Persteril), the concentration of active substance must be utaken into account when preparing the working solution. Inadequate (too low) concentration of the disinfectant used does not achieve a cidal effect, but only bacteriostatic, virostatic, and similar ones. Too high concentrations damage the disinfected objects and also lead to increased disinfection costs [9]. The pH values of the environment in which the disinfectant reacts with the microorganism is also an important factor affecting the final result. For example, glutaraldehyde and quaternary ammonium salts as well as chlorhexidine have higher efficacy at alkaline pH. On the other hand, phenolic preparations as well as chlorine are more effective at acidic pH. The temperature, in particular its increase, also partialy affects the end result of the disinfectant reaction with the microorganism [11]. At a low temperature of disinfectant solutions, dissociation of some disinfectants slows down, thereby reducing their diffusion into the bacterial cell. Some disinfectants (lyes) work better if they are heated to 70–80°C (in addition to chemical, they have physical effects too). Chloramine solutions are most effective at the temperature of 50–60°C [9]. The environmental pollutants of organic origin in which chemical disinfection is to be applied significantly reduce the activity of the disinfectant [11]. The presence of organic substances in the environment reduces the effect of all disinfectants; therefore, as previously mentioned before the disinfection of the object, it is necessary to clean the environment [9]. Microorganisms differ in their resistance to disinfectants. Different types of microoganisms vary in there responses to antiseptics, disinfectants, and sterilants (**Figure 1**). This is hardly surprising, in view of there different cellular structures, compositions, and physiologies. Tradititionally, microbial susceptibilities to biocides have been classified based on these differences. Bacterial spores are generally considered the organisms most resistant to antiseptics, disinfectants, and strilants, although prions have shown market resistance to many physical and chemical processes. It is important to note that this classification is considered only a general guide to antimicrobial activity and can vary depending on the biocide, formulation, or process under consideration [11].



	Microorganism	Examples
More Resistant	Prions	Scrapie, Creutzfeldt-Jakob disease, chronic wasting disease
↑	Bacterial spores	*Bacillus, Geobacillus, Clostridium*
	Protozoal oocysts	*Cryptosporidium*
	Helminth eggs	*Ascaris, Enterobius*
	Mycobacteria	*Mycobacterium tuberculosis, M. terrae, M. chelonae*
	Small, nonenveloped viruses	Poliovirus, parvoviruses, papillomaviruses
	Protozoal cysts	*Giardia, Acanthamoeba*
	Fungal spores	*Aspergillus, Penicillium*
	Gram-negative bacteria	*Pseudomonas, Providencia, Escherichia*
	Vegetative fungi and algae	*Aspergillus, Trichophyton, Candida, Chlamydomonas*
	Vegetative helminths and protozoa	*Ascaris, Cryptosporidium, Giardia*
	Large, nonenveloped viruses	Adenoviruses, rotaviruses
	Gram-positive bacteria	*Staphylococcus, Streptococcus, Enterococcus*
Less Resistant	Enveloped viruses	Human immunodeficiency virus, hepatitis B virus, herpes simplex virus

Figure 1.
Decreasing order of resistance of microorganisms to disinfection and sterilization and the level of disinfection or sterilization. source: McDonnell, 2007.

2.3 Biofilm

Biofilms are composed of immobilized bacteria deposited in an organic polymeric mass of bacterial origin. Biofilm cells are irreversibly connected to each other and with the surface via extracellular polymeric substances (EPS), which account for up to 85% of the total biofilm mass. EPS include a number of proteins, glycoproteins, glycolipids, and in some cases a surprising amount of extracellular DNA [12]. Extracellular polymers are produced by bacteria that allow bacteria to adhere to the surface. The polymeric products constitute the base matrix. Biofilm further contains substances that belong to the "*quorum sensing*" (QS) system that is involved in intercellular bacterial communication.

Biofilm formation is a dynamic process that includes the following steps:

- surface attachment
- the formation of a monolayer
- differentiation of microcolonies
- differentiation of macrocolonies and ultimately a mature form of biofilm being created [13]. Various nutrients in the wet environment absorbed on the surfaces create an acclimation coating with different physico-chemical properties. The physico-chemical properties of the surface determine how the bacteria attach. The structure of the biofilm is not homogeneous; it contains a set of numerous channels and cavities that serve to circulate water, supply nutrients and oxygen. They are intricate, mutually communicating channels of various shapes that supply substances and gases to biofilm-living bacteria. Bacteria in the biofilm form clusters of cells, which are known as microcolonies. Biofilm architecture is diverse, constantly changing in space and time due to external and internal processes. It is known that EPS production and hence the related biofilm thickness depend on the availability of nutrients and whether the biofilm is composed of one or more bacterial species. In a natural environment, the community of several species is more common. Biofilm formation is a cause of problems in many areas, e.g. in medicine, in water supply systems, in the food production industry [12]. Biofilm for microorganisms in a given environment creates both a nutritional layer that allows for their reproduction and a protective layer that limits the devitalizing effects of the disinfectants used. Thus, prevention is effective cleaning and application of combined compositions, e.g., oxidizing agents, surfactants and regular surface monitoring. The intervals needed; the intensity of cleaning and decontamination depend on the degree of contamination that occurs on different surfaces [14].

2.3.1 Methods of biofilm removal

The removal of impurities from surfaces must be of a high standard to ensure technological and hygienic requirements. In general, we distinguish three types of biofilm removal:

- mechanical
- chemical
- biological [15].

Physical methods (otherwise called mechanical methods) are based on the action of a magnetic field that is highly intense. Ultrasonic devices (high-frequency electric field devices) or their connection with organic acids are used. Chemical methods consist of using detergents and disinfectants the acting of which is necessary to effectively remove biofilms. Significant is the use of ozone and a variety of chlorine-based preparations, iodine compounds, peroxyacetic acid, and quaternary ammonium compounds [13]. Biological methods degrade biofilms using enzymes produced by bacteria, but their use is limited because of their cost and affordability. In order to achieve the desired effect, it is appropriate to use a combination of methods with a synergistic effect.

2.4 Chemical disinfection

Disinfectants are classified by their chemical nature and each class has its unique characteristics, hazards, toxicities and efficacy against various microorganisms. Environmental conditions, such as the presence of organic matter, pH or water hardness can also impact the action of the disinfectant [10].

Different disinfectants have different mechanisms of action, all disinfectants act by harming microorganisms in some manner. Mechanisms of harm include:

1. Protein denaturation
2. Membrane disruption
3. Nucleic acid damage
4. Inhibition of metabolism [16].

The ideal disinfectant must have a wide range of action, rapid, time-saving use, and water solubility. The disinfectant should also be stable, ecological, non-toxic, non-corrosive, economical and safe to use. However, such a disinfectant does not exist in real practice and it is therefore important to choose a suitable combination of disinfectants. Rotation of disinfectants is good practice. The mechanism of action of disinfectants on microorganisms can generally be divided into four categories:

- denaturation of proteins
- membrane damage
- damage of nucleic acids
- inhibition of metabolic activity.

Disinfectants are placed into three categories depending on microbicidal activity:

- High-level disinfectants
- Intermediate-level disinfectants
- Low-level disinfectants [17].

High-level disinfectants (HLD) are active against vegetative bacteria, viruses (including the nonenveloped ones), fungi, and mycobacteria. They may also have some activity against bacterial spores with extended contact times. Aldehydes (glutaraldehyde and ortho-phthalaldehyde) and oxidisers (e.g., hydrogen peroxide and peracetic acid) are HLDs. The aldehydes are non-corrosive and safe for use on most devices. However, they can fix organic materials; therefore, it is particularly important to remove any embedded microbes prior to disinfection. Unless properly formulated and carefully used, oxidisers can be corrosive. However, they can be faster-acting, non-fixative, and safer for the environment than aldehydes. HLDs typically require 10–45 min of contact time for disinfection, depending on the temperature. After disinfection, items require thorough rinsing with sterile or filtered water to remove any chemical residues; they must then be dried with an alcohol rinse or by blowing clean and filtered air through the device's channels prior to safe storage. A disinfectant (e.g., ethanol) is active against vegetative bacteria, mycobacteria, fungi, and most viruses. It may fail to kill spores, even after prolonged exposure. Low-level disinfectants (e.g., quaternary ammonium compounds) are active against vegetative bacteria (except mycobacteria), some fungi, and only enveloped viruses. In many cases, washing with unmedicated soap and water would be sufficient in place of such disinfectants [18]. There are three levels of disinfection: high, intermediate, and low. High-level disinfectants, such as glutaraldehyde, are used as chemical sterilants and should never be used on environmental surfaces. Intermediate-level disinfectants are registered with the Environmental Protection Agency (EPA) and have a tuberculocidal claim, and low-level disinfectants are EPA-registered without a tuberculocidal claim (i.e., hepatitis B virus and HIV label claims). The process of high-level disinfection, an appropriate standard of treatment for heat-sensitive, semicritical medical instruments (e.g., flexible, fiberoptic endoscopes), inactivates all vegetative bacteria, mycobacteria, viruses, fungi, and some bacterial spores. High-level disinfection is accomplished with powerful, sporicidal chemicals (e.g., glutaraldehyde, peracetic acid, and hydrogen peroxide) that are not appropriate for use on housekeeping surfaces. These liquid chemical sterilants/high-level disinfectants are highly toxic. Use of these chemicals for applications other than those indicated in their label instructions (i.e., as immersion chemicals for treating heat-sensitive medical instruments) is not appropriate [17]. Intermediate-level disinfection does not necessarily kill bacterial spores, but it does inactivate *Mycobacterium tuberculosis* var. bovis, which is substantially more resistant to chemical germicides than ordinary vegetative bacteria, fungi, and medium to small viruses (with or without lipid envelopes). Chemical germicides with sufficient potency to achieve intermediate-level disinfection include chlorine-containing compounds (e.g., sodium hypochlorite), alcohols, some phenolics, and some iodophors [18]. Low-level disinfection inactivates vegetative bacteria, fungi, enveloped viruses, e.g., human immunodeficiency virus (HIV) and influenza viruses, and some non-enveloped viruses (e.g., adenoviruses). Low-level disinfectants include quaternary ammonium compounds, some phenolics, and some iodophors [2]. The health and safety of humans and animals should always be a primary consideration when selecting a disinfectant. Most disinfectants have some level of hazard associated with their use. Some pose a serious threat to human and animal health (i.e., aldehydes, phenols, sodium hydroxide). Some cannot be used when animals are present or must be thoroughly rinsed away with potable water prior to restocking. Personnel training, personal protective measures and safety precautions should always be taken. Environmental factors, such as runoff into creeks or ponds, must also be considered when selecting a disinfectant. Many agents are known ecological hazards for plants and aquatic life (i.e., sodium carbonate, hypochlorites, phenolic compounds), therefore drainage, runoff, and biodegradability of disinfectants should be considered [19].

2.4.1 Chemical disinfectants

Chemical disinfectants are chemical agents applied to non-living objects in order to destroy bacteria, viruses, fungi, mold or mildews living on the objects. By definition, disinfectant formulas must be registered with the Environmental Protection Agency (EPA). The "active ingredient" in each disinfectant formula is what kills pathogens, usually by disrupting or damaging their cells [20].

2.4.1.1 Alkalis

Alkalis (or bases) are defined as substances capable of forming hydroxide (OH^-) ions when dissolved in water and are measured at $pH > 7$. Hydroxides are strong bases with a pH above 12 and are very reliable disinfectants. Alkalis have good microbicidal properties; inhibit the growth of microorganisms by restricting various metabolic processes. In general, pH values of ≥9 are restrictive for the growth of most vegetative microorganisms, including bacteria and fungi. Low concentrations are generally inhibitory, while higher concentrations are bactericidal and fungicidal. Typical virucidal concentrations are 1–2% NaOH [21]. The mechanism of action of the hydroxide is based on changing the pH of the environment. The reaction of alkali with the various types of lipids (including phospholipids) in these membranes can be compared to their reactions with fatty acids in lipids and oils to cause salt (soap) formation. Membrane disruption leads to cell wall destabilization and loss of membrane structure and function, including disruption of the proton motive force and leakage of cytoplasmatic materials. Alkali also causes breakage of peptide bonds and the breakdown of proteins, which is presumed to be the major mechanism of action against prions [22]. Alkalis are very corrosive agents and damage to various surfaces, depending on the concentration of alkali used and the formulation pH. Personal protection precautions should be observed while working with alkalis [21]. Some limited disinfection methods use high concentrations of strong alkalis, such as NaOH (commonly known as caustic soda or soda lye) and KOH (also known as lye), while lower concentrations of these and weaker alkalis, such as sodium bicarbonate (baking soda) and sodium matasilicate, are used in various cleaning applications [23].

Potassium hydroxide (KOH) is used to produce greasy antiseptic soaps [24].

Sodium hydroxide (NaOH) is a strong surface disinfectant which finds a use in many farm situations [21]. The disinfecting effect of the lye depends on the concentration of hydroxyl ions. Sodium hydroxide has a moderately wide range of action. At concentrations of 3–5%, it has bactericidal effects, especially on gram-negative rods. The effect on cocci is not sufficient. Already at a 2% concentration it has a good virucidal effect on most viruses. Sodium hydroxide does not act well on mycobacterial rods and fungi [24]. A high concentration of this substance can kill all microorganisms including bacterial spores. Such concentrations will produce a pH of 13 or higher [21]. NaOH is stored in well-closed containers because it reacts with CO_2 in the air and thus loses efficiency, so freshly prepared hydroxide solutions should be used. It is well soluble in hot water (e.g., in water 18°C warm it dissolves to 51%, but in water heated to 70–80°C up to 75%), producing heat, as a side effect. It dissociates in water into negatively charged hydroxyl ions and positively charged sodium ions. Sodium hydroxide is highly corrosive and irritating to the skin, eyes and mucous membrane s of animals and humans; contact could result in severe burns. Most problems occur after careless use of this disinfectant [21]. Sodium hydroxide is a corrosive with a good deep effect [24]. Extreme caution is required when handling NaOH. Great care must be taken regarding the environmental impact of this product, especially when dealing with water run-off, as sodium hydroxide may severely affect the pH of surface water and plant life. It is recommended that this disinfectant be used only when there is absolute certainty that the environment will not be negatively affected. However, NaOH has the advantage of being relatively cheap and lends itself to being handled in bulk [21].

Calcium hydroxide Ca(OH)2 is prepared from burnt lime by slaking with water. A 20% suspension (lime milk) is prepared from freshly slaked lime. Slaked lime absorbs air carbon dioxide and turns into calcium carbonate, which is ineffective as disinfectant. The suspension prepared from freshly slaked lime has both viral and bactericidal effect [9].

2.4.1.2 Acids

Acids are defined as substances that dissociate in water to provide hydrogen ions (H^+), which are measured on the pH scale as <7 [25]. The effect of acids and their derivatives is based on the action of hydrogen ions, anions or whole molecules, surface activity, oxidative or dehydrating capabilities. Acidic disinfectants function by destroying the bonds of nucleic acids and precipitating proteins [21]. Acids also change the pH of the environment of cell, cause oxidaze a dehydratation as well as the destruction of fermentative metabolism of bacteria [26]. The effectiveness of the acids is reduced by the presence of organic contamination. Disadvantages of organic acids are their ability to interact with organic substances, thereby reducing their disinfectant activity, etching and corrosiveness [27]. The use of inorganic acids is considerably limited due to their corrosive and irritant effects. Of inorganic acids, hydrochloric acid, nitric acid and phosphoric acid are used in the disinfection practice [9].

Hydrochloric acid (HCl) is used in the form of *Schattenfroh solution*. The solution contains 2.5% hydrochloric acid and 15% cooking salt. It is used to disinfect anthrax – contaminated skin [24].

Nitric acid (HNO3) has a good sporocidal effect. It is used at a 2% concentration for bristle disinfection at 2 h exposure and a solution temperature of 40°C. After disinfection is complete, the bristles are neutralized with a 2% sodium hydroxide solution. A concentration of 0.3–0.5% at a solution temperature of 50°C is recommended today mainly for cleaning and disinfection of milking equipment in organic farming [28].

Phosphoric acid (H3PO4) is used to disinfect soil and manure at a concentration of 1.5–3%. Of the organic acids, peracetic acid and lactic acid are used in disinfection practice.

Peracetic acid (CH3COOH) is the most potent of the above-mentioned substances, acting in a bactericidal, sporocidal, viricidal and fungicidal way. Peracetic acid is part of Persteril, a composition which contains 32–36% peracetic acid, 7–10% hydrogen peroxide, 1% sulfuric acid. Persteril is an unstable preparation and is prepared for the active substance content [29].

Peracetic acid is oxidizing agents, denatures proteins, disrupts cell wall permeability, and oxidizes sulfhydral and sulfur bonds in proteins, enzymes, and other metabolites [27]. Its advantage is that it works at low concentrations. At a concentration of 0.4%, it acts on the surfaces after a 30 min exposure. Persteril as a 0.1% solution is used to treat growing mold directly on the meat. For hand disinfection it is used as a 1–0.2% solution. It leaves no residue, rapidly decomposes into acetic acid and water. Peracetic acid is used to disinfect the environment, surfaces and medical devices. In the form of an aerosol or spray, Pedox-PAA50, which contains 10–40% peracetic acid, is the most commonly applied formulation. For aerosol disinfection, it is used in the concentration of 5–7 ml m^{-3}. The disadvantages of using peracetic acid include corrosion to metals. Even this disadvantage can be avoided by the addition of sodium pyrophosphate in the ratio 1:2 to peracetic acid. It has to be stored at a temperature below 20°C. It is best stored in a refrigerator at 4°C [29].

Lactic acid is mainly used to disinfect air in the presence of animals. It is used in the form of an aerosol, in an amount of 5 ml m^{-3} [30].

2.4.1.3 Halogens

Halogen-containing disinfectants include chlorine, iodine, bromine and fluorine preparations, which are the most reactive and the most toxic of the halogen compounds. Halogen-containing compounds which are toxic to the cell are created by the action of oxygen in the initial phase. The optimum pH for the disinfection effect is 5–8 and the presence of organic substances significantly reduces it. For practical disinfection, iodine, chlorine and its compounds are important [31].

2.4.1.4 Iodine and iodonal

The position and importance of iodine among the disinfectants lies in its intense and, above all, rapid action on all microorganisms at quite low toxicity. Iodine is a crystalline substance that sublimes at normal temperature and pressure. However, aqueous or alcoholic iodine solutions carry many undesirable effects and their wider use in disinfection was hindered by their significant negative properties such as low solubility in water, corrosion, staining of disinfected objects, toxicity, and the like [9]. Iodine-based disinfectants are called iodophores. Iodophores are relatively non-toxic. In iodophores, iodine is bound to polyvinylpyrrolidones (surface-active organic polymers), which have a significant effect on increasing the disinfection efficiency of these formulations.

Iodine compounds are broad spectrum and considered effective for a variety of bacteria, mycobacteria, fungi and viruses [23]. The negative properties of iodophores are considerably limited, have corrosive effects on iron, less affect copper and its compounds. They have a weak corrosive effect on zinc, aluminum and tin. They do not rust stainless steel. At long-term use, they leave stains and color PVC (polyvinyl chloride) and polyethylene. Iodophores are water-soluble, stable, non-allergenic, fast-acting, low-toxicity, and non-irritating to injured skin. When using iodophores, the basic requirement, namely a thorough mechanical cleansing, must be fulfilled. The temperature of the solutions should not exceed 35°C [32]. They are used in healthcare, veterinary care, food production industry, agriculture and municipal hygiene. Iodine preparations can be used both to disinfect surfaces and to disinfect skin as antiseptics.

Jodonal A contains 1.75% active iodine, 12.5% phosphoric acid and a stabilizer. It has viracidal, sporocidal and bactericidal effects, also against acid-resistant mycobacteria. It is used in the food production industry.

Jodonal B contains 1.65% active iodine, 3.6% phosphoric acid and a stabilizer. Jodonal B is used in health care and municipal hygiene.

Jodonal M contains 1.6% iodine, followed by citric acid and glycerin, which has a protective effect on mucous membranes. Jodonal M is used in the prevention of mastitis in cows, is designed for disinfection of teats after milking at 20% concentration. It is used for udder treatment in 2–4% concentration [9].

2.4.1.5 Chlorine and its compounds

Chlorine preparations are widely used. Chlorine is responsible for the major mechanism of action and thus the inactivation of enzymes and ribosome proteins, due to the formation of a strong oxidizing agent – HClO (hypochlorous acid), which is the result of the reaction of chlorine with water. The bacterial cell undergoes changes in the cytoplasmic membrane, the oxidation of thiol groups of enzymes and chlorination of nucleotides occurs, resulting in the blockade of DNA synthesis [33]. An important element is chlorine, occurring in the form of poisonous yellow-brown gas. Chlorination is the most widely used method for disinfecting water supplies. The disinfecting ability of chlorine in water depends on the degree of its dissociation. In an acidic environment, their disinfection effect increases. Chlorine preparations contain salts of hypochlorous acid (HClO). Their decomposition in aqueous environment produces hydrochloric acid (HCl) and oxygen in the phase of "birth," which oxidizes organic substances. The chemical activity of the chlorine preparations is associated with the chlorine found together with the oxygen in the hypochlorite group -ClO. The amount of oxygen released by the decomposition of this group corresponds to the content of reactive chlorine in a preparation called as active chlorine. Thus, active chlorine is an indicator of disinfectant properties in chlorine preparations. The active chlorine content in the chlorine preparations is expressed as a percentage. Chlorine preparations belong to the group of oxidizing agents with very good disinfection effect [34, 35]. Chlorine compounds are inactivated by organic soil, so a cleaning step is often required for heavily soiled surfaces. They are also prone to degradation from exposure to heat, UV light, and transition metals, such as copper, nickel, cobalt, and iron [36]. Activated solutions are recommended for disinfection especially for mycobacteria and spore-forming bacteria. In the food producing industry, it is not recommended to disinfect the surfaces with which the raw materials or food come into direct contact with chlorine preparations. The effect of all chlorine derivatives is accelerated by the addition of ammonia and ammonium salts, which is the essence of so-called activation of chlorine preparations. However, this activation is short-lived so that the activated solutions must be used immediately, especially against the highly resistant microbes. Ammonium salts, in the ratio 1:1 and ammonia, in the ratio 1:8 to 16 [9] are added to the solutions of known concentration. The most commonly used chlorine preparations include chloramines, chlorinated lime, dikonit, sodium hypochlorite.

Chloramines are organic compounds containing 25–30% active chlorine. Chloramines are stable powder substances, well soluble, with corrosive and whitening effects. They have bactericidal, fungicidal and virucidal activity. At alkaline pH, their effect decreases rapidly. In disinfection practice, Chloramine T is the most significant. In aqueous solution it hydrolyses more slowly than chlorinated lime, explaining its more gentle action on fabrics, metals, wood and other disinfected materials. Chloramine T is a relatively stable preparation. Losses of active chlorine when stored correctly are only 0.1% per year. Chloramine T solutions heated up to 50–60°C are more effective than non-heated solutions. Chloramine T has a bactericidal effect, while higher concentrations (10%) are required for mycobactericidal action in practical disinfection. Preventive disinfection uses a 2–3% solution, 4–10% for focal disinfection [9].

Fresh chlorinated lime contains 33–36% of active chlorine. It dissolves only partially in water. It is unstable in air and must be stored in impermeable, well sealed containers, dry and protected from light. The effectiveness of chlorinated lime is reduced by storage. It is used for wastewater disinfection, coarse disinfection and 2–3% for floor disinfection at 30 min. Exposure or till dry. Chlorinated lime has a good bactericidal effect, also against acid-resistant mycobacteria, further sporocidal effect, which can be enhanced by acidification with sulfuric acid, as well as a good virucidal activity. For practical disinfection, cold clarified solutions containing 1–2% of active chlorine are prepared. Chlorinated lime is used to disinfect farm buildings, cowsheds, paddocks, fences and cesspools [37].

Dikonit is a granular highly effective disinfectant preparation of chlorinated cyanuric acid containing at least 55% of active chlorine. It dissolves well in water. The solid surfaces are disinfected with 1–2% solution. Dikonit has the widest use in health care and community hygiene. In terms of its effect on microorganisms, it has bactericidal, fungicidal, virucidal, tuberculocidal effect.

Other representatives include **sodium hypochlorite**. Sodium hypochlorite is also called “liquid chlorine”, which has bactericidal and virucidal properties.

2.4.1.6 Aldehydes

Aldehydes are highly effective, broad spectrum disinfectants, cause against bacteria, fungi, viruses, mycobacteria and spores [23]. The mechanism of action of aldehydes is based on protein denaturation and disrupting of nucleic acids [38]. The most commonly used agents are formaldehyde and gluteraldehyde. Aldehydes are non-corrosive to metals, rubber, plastic and cement [39]. These chemicals are highly irritating, toxic to humans or animals, therefore their use is limited [40].

Formaldehyde is an irritating gas dissolving in water into a nearly 40% solution called formalin. It has excellent microbicidal effects, reliably destroys bacteria, spores, fungi and viruses. It is used for surface disinfection at 2–3% concentration. It is also used as an aerosol. In special devices, formaldehyde mixed with water vapor at 60–80°C can be used to sterilize some instruments and delicate items. The disadvantage is its irritating odor almost to toxicity [10]. Formaldehyde has been classified by the International Agency for Cancer Research as a Group 1 Carcinogen, i.e. a proven human carcinogen. In view of the carcinogenic and teratogenic effect, formaldehyde used for a long time has been restricted in use, but is still used for sterilization in chemical autoclaves; however, it must be ensured that its vapors are sucked out of the environment so that the operator of the device is protected.

Glutaraldehyde is primarily used as a disinfectant for medical equipment (e.g., endoscopes), but can provide sterilization at prolonged contact times [38]. Glutaraldehyde has a broad spectrum of activity against bacteria and their spores, fungi, and viruses. Bacterial studies demonstrated a strong binding of glutaraldehyde to outer layers of organisms such as *E.coli* and *Staphylococcus aureus* [21]. A 2% concentration is used for highlevel disinfection. Its efficacy is highly dependant on pH and temperature, working best at a pH greater than 7 and high temperatures. It is considered more efficacious in the presence of organic matter, soaps and hard water than formaldehyde [38, 40].

2.4.1.7 Alcohol

The most feasible explanation for the antimicrobial action of alcohol is denaturation of proteins. Protein denaturation also is consistent with observations that alcohol destroys the dehydrogenases of *Escherichia coli*, and that ethyl alcohol increases the lag phase of *Enterobacter aerogenes* and that the lag phase effect could be reversed by adding certain amino acids. The bacteriostatic action was believed caused by inhibition of the production of metabolites essential for rapid cell division [21].

2.4.1.8 Surfactants

Surfactants from the Latin "*tensio*" are surface active substances that reduce the surface tension of liquids. According to the polar group, surfactants are divided into two basic groups, ionogenic and non-ionic. Ionogenic surfactants contain functional groups that dissociate in aqueous solution, thereby producing negative (anionic) or positive (cationic) charged ions. Their charge depends on the pH of the environment. Non-ionic surfactants are substances that do not dissociate in aqueous solution. Anionic surfactants include detergents and sulfonate detergents. Cationic surfactants have a bactericidal effect in addition to cleaning and wetting properties [41]. They act better in the alkaline environment, they are not corrosive and do not irritate the skin, also non-toxic, colorless, odorless and stable in the presence of organic material. Quaternary ammonium compounds are the most important class of surfactants that exhibit strong disinfectant effects. The best-known preparations are Ajatin and Septonex [9].

Ajatin is an effective disinfectant that acts on vegetative bacteria, the disadvantage of which is its low potency against spores and tuberculosis agents. It is used in 1% concentration for hand disinfection and in 5% concentration for skin disinfection. If we increase its concentration to 10%, it can only wash the hands for 3 min. The action of Ajatin consists in disrupting bacterial membranes and structures, inhibiting the metabolism of bacteria and causing denaturation of proteins and enzymes.

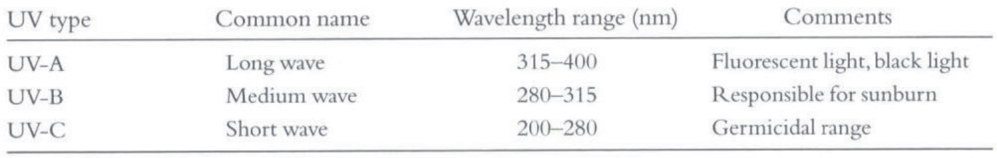
Septonex is a white powder, used as a 1% solution for hand, object and laundry disinfection [42].

2.5 Physical disinfection

Physical disinfection is based on the effect of physical quantities on the pathogenic microorganism. One of the variables is the exposure time, which precisely determines the time interval during which another physical quantity (temperature, wavelength, etc.) must act.

2.5.1 UV radiation

Ultraviolet germicidal radiation is an established means of disinfection and can be used to prevent the spread of certain infectious diseases. UV radiation is used to control airborne microorganisms and environmental surface decontamination [39]. The main sources of UV radiation are simple UV lights, including mercury vapor lamos, fluorescent lights, pulsed UV lamps, and "black – light" lamps [21]. Not all UV wavelenghts are effective against microorganisms (**Figure 2**). A main characteristic of UV light is that a specific range of its wavelengths, those between 200 and 300 nm, are categorized as germicidal, they are capable of inactivating bacteria, viruses and protozoa. This capability allows widespread adoption of UV light as a chemical-free, environmentally friendly and highly effective way to safeguard and disinfect water against harmful microorganisms [43]. The most effective



UV type	Common name	Wavelength range (nm)	Comments
UV-A	Long wave	315–400	Fluorescent light, black light
UV-B	Medium wave	280–315	Responsible for sunburn
UV-C	Short wave	200–280	Germicidal range

Figure 2.
Types of UV radiation. Source: McDonnell, 2007.

wavelenght has been found to be 265 nm [21]. Unlike chemical approaches to water disinfection, UV provides effective and rapid inactivation of microorganisms through a physical process. Inactivation by UV light act through the direct absorption of UV energy by the microorganism, causing a molecular rearrangement of one or more of the biochemical components that are essential to the organism's functioning. Microorganisms are inactivated by UV light as a result of damage to nucleic acids. The high energy associated with short wavelength UV energy, primarily at 254 nm, is absorbed by cellular RNA and DNA, this absorption forms new bonds between adjacent nucleotides, creating double bonds or dimers. Dimerization of adjacent molecules, particularly thymine, represents most common photochemical damage. Formation of thymine dimers in the DNA of viruses and bacteria prevents replication and inability to infect. UV light demonstrate efficacy against pathogenic organisms, including those responsible for typhoid, hepatitis, cholera, polio and other viral, bacterial and parasitic diseases [11]. Benefits of UV:

- UV produces no residual
- UV requires no transportation, storage or handling of toxic or corrosive chemicals – a safety benefit for plant operators and the surrounding community
- UV treatment creates no carcinogenic disinfection by-products that could adversely affect quality of the water
- UV is highly effective at inactivating a broad range of microorganisms – including chlorine-resistant pathogens like *Cryptosporidium* and *Giardia*
- UV can be used (alone or in conjunction with hydrogen peroxide) to break down toxic chemical contaminants while simultaneously disinfecting.

UV offers a key advantage over chlorine-based disinfection, due to its ability to inactivate protozoa that threaten public health – most notably *Cryptosporidium* and *Giardia.* The release of these harmful microorganisms into lakes and rivers by wastewater facilities utilizing chlorine disinfection increases the potential of contamination in communities that rely on these same bodies of water for their drinking water source and recreational use. Drinking water treatment plants can benefit by using UV since it can easily inactivate chlorine-resistant pathogens (protozoa), while reducing chlorine usage and by-product formation [43]. In addition, UV light, either alone or in conjunction with hydrogen peroxide can destroy chemical contaminants such as industrial solvents, pesticides and pharmaceuticals through a UV-oxidation [11]. Safety is a major concern since UV radiation can cause severe eye damage and skin irritation of exposed individuals. Furthermore, bacterial regrowth may occur because there is no residual antimicrobial activity. When exposed to visible light, bacterial cells that had been injured by UV light can repair themselves [44].

2.5.2 Ozone disinfection

Ozone is a very powerful disinfectant, unstable gas that can destroy bacteria and viruses. Is one of the strongest oxidation agents. It is an air pollutant of much concern in Europe, because it can affect human health and damage the environment. Because of its short half-life, ozone decay soon when produced. The half life of ozone in water is about 30 min, which means that every half hour the ozone concentration is reduced to half its initial concentration. In practice the half-life is shorter because a lot of factors (temperature, pH and concentration) can influence the half-life. Because ozone reacts with all kinds of components, the concentration ozone reduces quickly. The word ozone, from the Greek "*ozein*" [43], was first used in 1840 by the chemist C. F. Schonbein [45, 46], a professor at the University of Basel who sensed that, by subjecting oxygen to electric shock, a strange smell was emitted into the air, due to the presence of a gas called ozone. Ozone is a more effective disinfectant than chloramines, chlorine, and chlorine dioxide. Several studies proved that ozone, unlike chlorine products, can deactivate resistant micro-organisms. Although chlorine is very suitable for the deactivation of bacteria and viruses, it cannot be used to deactivate protozoa. Protozoa spread through the environment rapidly as cysts, which live longer and are more resistant to disinfectants than bacteria or viruses. In general, *Cryptosporidium parvum* causes larger problems for drinking water treatment than *Giardia Lambia* does. *Cryptosporidium parvum* is 4–5 μm in size, which makes it difficult to remove by conventional filtration [47]. *Giardia Lambia* is 8–14 μm in size, which makes it easier to remove by conventional filtration than *Cryptosporidium parvum* [17]. Moreover, when drinking water or swimming pool water is treated by means of conventional chlorination, *Cryptosporidium parvum* is insufficiently deactivated. Alternative treatment methods, such as ozone and UV disinfection, appear to have the ability to deactivate the oocysts sufficiently [33, 48]. The benefit of ozone is that it influences temperature and pH minimally on a broad spectrum. Ozone solubility decreases when temperatures rises, disinfection rates increase per 10°C. Within the range of 0–30°C, these two factors diminish one another. The disinfection rate of ozone hardly changes in a pH range of 6–8.5 [49]. For certain resistant microorganisms (such as *Giardia Muris*), the disinfection rate increases at higher pH values [10, 34], but for other species of microorganisms, this is the other way around. Other benefits of ozone application are:

- No remaining tastes or odors after treatment.
- Disinfection byproduct formation is minimal.
- Ozone can remove disinfection byproduct precursors.

Ozone is not always the most suitable disinfectant. Ozone is less suitable for maintenance of a residual concentration, causing it to decompose in water relatively quickly [49]. Chlorine is more suitable for residue formation [10].

2.5.3 Ultrasound

Ultrasound refers to inaudible sound waves with frequencies in the range of 16 kHz–500 MHz, greater than the upper limit of human hearing. It can be transmitted through any elastic medium including water, gas-saturated water, and slurry. Ultrasound has been used for diverse purposesin many different areas. In water treatment technology, the application of ultrasound (ultrasonication) can be useful in various processes like organic decontamination, disinfection, electrocoagulation, and membrane filtration. Because of cavitation phenomenon, the formation of free radicals and high localized temperatures and pressures, ultrasonic irradiation (ultrasonication) appears to be an effective method for the destruction of hazardous organic compoundsin water [50]. These compounds include phenol [51] chlorophenols, nitrophenols, aniline [52], trichloroethylene [53], ethylbenzene [54], chlorobenzene [55], chloronaphthalene, polychlorinated biphenyls, pesticides, polycyclic aromatic hydrocarbons, azobenzene, textile dyes [56], carbofuran, nitroaromatics, detergents and surfactants [57]. High power ultrasound, operatedat low frequencies is an effective means for disintegration of bacterial cells. However, disinfection by ultrasonication alone requires very high energy. Thus, generally it cannot be considered as an alternative to conventional disinfection for economical aspects. Then, ultrasonication should be used together with other techniques. For instance, the combination of a short ultrasonication and a subsequent ultraviolet treatment is even cost-efficient and meaningful [58]. Ultrasonication combined with chlorination improved significantly the biocidal action. These results suggest that ultrasound could be used in conjunction with chemical treatments to achieve a reduction in the quantity of bactericide required for water treatment [59]. Ultrasound irradiation can provide enhancement in membrane filtration of waste waters [60]. It increases the flux primarily by breaking the cake layer at the membrane surface. Liquid jets produced by cavitation served as a basis for ultrasonic membrane cleaning. Lower ultrasound frequencies have higher cleaning efficiencies than higher frequencies [61]. Intermittent ultrasound ir-radiation resulted in the same flux obtained as continuous irradiation but intermittent ultrasound consumed less energy and prolonged the lifetime of the membranes used, thus can be considered as a cost effective method of membrane cleaning [48]. Ultrasound can produce various effects on biological materials, for example, stimulating enzyme activity, cell growth, biosynthesis, etc., which enhances the bioactivity of the activated sludge. Thus, the improvement in efficiency of enhanced biological removal of phosphorus [62] and nitrogen [63]. Low frequency (25 kHz) was more effective than higher ones (80 and 150 kHz), or in other term, higher energy ultrasound was more efficient than lower energy ultrasound for the sludge treatment, indicating that mechanical effects, instead of free radicals, were responsible for the bioactivity enhancement [63]. Comparing with other pre-treatment methods, ultrasonication exhibits a great potential of not being hazardous to environment and for being economically competitive [64]. Ultrasound is used in the remediation of contaminated soil and sediment [47]. Ultrasonic leaching has been investigated for the decontamination of different types of soils from landfills, mining spills, and river sediments aswell as various types of contaminants like organic compounds. The application of ultrasound in air pollution control is based on acoustic agglomeration phenomenon that makes small particles precipitated for easy removal. Acoustic agglomeration is a process in which high intensity sound waves produce relative motion and collisions among fine particles suspended in gaseous media. Acoustic agglomeration can be conducted in two approaches, with low frequency and high frequency (ultrasound) sonication. While low frequency acoustic field is more cost and energy efficient, high frequency acoustic (ultrasonic) agglomeration might achieve better particle retention efficiency, especially for very small particles in submicron range [49].

3. Conclusion

Almost every environment on the planet contains microorganisms. Sanitation represents an applied science because of its importance to the protection of human health and its relationship with environmental factors that relate to health. This applied science relates to control of the biological, chemical, and physical hazards in a environment. Effective sanitation practices are needed to combat their proliferation and activity. Appropriate choice of disinfectant, setting clear goals and a reliable action plan are necessary steps to ensure the safety of animals, people, equipment and the environment.

Acknowledgements

The work was supported by the project VEGA 2/0125/17.

Conflict of interest

The authors declare no conflict of interest.

Abbreviations

pH	potential of hydrogen
HLD	high-level disinfectants
EPA	environmental protection agency
HIV	human immunodeficiency virus
OH^-	hydroxide ions
CO2	carbon dioxide
%	percentage
°C	degree Celsius
NaOH	sodium hydroxide
CaOH	calcium hydroxide
H^+	hydrogen ions
HCl	hydrochloric acid
HNO_3	nitric acid
H_3PO_4	phosphoric acid
CH_3COOH	peracetic acid
$ml\ m^{-3}$	milliliter per cubic meter
ClO^-	hypochlorite
PAL	surface active substances
UV	ultraviolet
nm	nanometer
RNA	ribonucleic acid
DNA	deoxyribonucleic acid
kHz	kilohertz
MHz	megahertz
EPA	environmental protection agency
EPS	extracellular polymeric substances
QS	quorum sensing

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