# Microbiological challenges to the pharmaceuticals and healthcare

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## 22.1 Introduction

Microorganisms may be introduced into pharmaceuticals via the materials used in their manufacture and through various environmental sources during processing. Once microorganisms are present, proliferation may occur if conditions are favorable. With sterile products, any contamination presents a risk to the patient. With nonsterile products, an assessment of risk is more complex. Here the absolute numbers and organism types are key factors that need to be taken into account.

To minimize risk, there are key control points that can effectively limit bioburden (the number of microorganisms present in or on a material) and control microbial growth. To explore that the suitable control points are in place and operating correctly, a comprehensive biocontamination control strategy needs to be in place. This will center on limiting the potential for contamination during manufacture.

This chapter takes an overview of pharmaceuticals and healthcare in relation to the different sources from which microorganisms might contaminate a pharmaceutical product. The fate of these contaminant organisms is considered together with the consequences of their survival and growth upon the product and, consequently, for the consumer or patient. In taking this overview, the chapter ties together the main themes of the book and places them within the context of ensuring patient and consumer safety. In doing so, the chapter also re-emphasizes the essential role of the pharmaceutical microbiologist [1].

## 22.2 Microbial risks to pharmaceuticals

The risk to people from spoiled or otherwise adulterated pharmaceuticals has been appreciated for many centuries. However, it was only during the late nineteenth and the early twentieth centuries that the particular role played by microorganisms in this process was understood. Even here, the steps taken to minimize the risk of contamination were relatively gradual, and these steps have progressed in tandem with increased knowledge. For example, it was not until the 1970s that cleanrooms, originally conceived for the development of nuclear weapons, became commonplace within pharmaceutical facilities in order to provide a clean air barrier to protect the product.

Microbial contamination and spoilage of pharmaceuticals will not only alter the esthetic qualities of a product (color, smell, texture, and so forth); such contamination may also render the product dangerous to the user; or it may also nullify any intended therapeutic value of the product. Here the infection risk presented by pharmaceuticals

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varies according to the route of application of the product, the health status of the user and the nature of the contaminating microorganism (to take some of topics first raised in Chapter 2). Thus, those products which are injected directly into blood vessels or tissues (injections and infusions), and those that are applied directly to the eyes and ears (contact lens solutions, eye drops, etc.) represent a greater infection risk than products that are taken orally or applied to intact healthy skin. The infection risks from injected products and eye products are sufficiently great that all such products must be manufactured in such a way that they are completely free from all types of microorganism (i.e., they are classed as "sterile products"). In contrast, oral and topical products may contain a small number of certain types of microorganisms (excluding specific pathogens) [2].

The assessment of pathogenic microorganisms is important for the risk assessment of nonsterile products, and, in general, monitoring microbial distribution and identifying the predominant isolates are part of good manufacturing practices [3]. Those organisms assessed as dangerous to the patient must be assessed and specified. Although indicator organisms can be used, and it is sensible to include such organisms as a part of method qualification, the list of organisms must be based on what could actually present a risk to the product or process. For such analyses, phenotypic or genotypic microbial identification systems are required [4].

In relation to the patient population, individuals who are immunodeficient, either through clinical disease or through use of immunosuppressive drugs are at a greater risk of infection than young healthy individuals. Damaged skin (burns, cuts, spots, etc.) is more easily infected than healthy intact skin, and special care must, therefore, be taken with products that might be applied to broken or burnt skin.

## 22.3 Microbial challenges to process environments

The environment where pharmaceuticals and healthcare products are prepared presents a potential vector for contamination (as Chapter 16 demonstrates). With environment, examination of air for microbial content can be demonstrated by exposing dishes containing agar media directly to the air (settle plates) or through drawing in a fixed volume of air through a device that can capture a proportion of the microorganisms in the air-stream (volumetric air-samplers) [5]. Microorganisms falling onto the surface of the agar or deposited upon it produce visible colonies on incubation.

It is not straightforward to generalize about the number of microorganisms present in air, since this varies markedly with location. Air samples taken from within occupied buildings will tend to have a greater bacterial content than those taken outdoors; however, outside fungal cells predominate over bacteria. Typically, total microbial counts of air, taken from occupied rooms, vary between 10 and $10^4$ microorganisms per liter. High values are likely to be associated with activities such as milling, hay-making, and so forth. In an uncontrolled laboratory environment, counts typically fall between 100 and 500 cells per liter. Numbers would be appreciably smaller for a cleanroom environment with adequate air filtration. This descending level of contamination tends

to run to a standard pattern for areas that are unoccupied (or "at rest," to draw upon cleanroom terminology) [6].

Microbial numbers rise with increasing numbers of people in the room and also with the degree of physical exertion that they are undergoing. The wearing of appropriate protective clothing will do much to contain the shedding of skin scales and bacteria to the air. Further protective measures arise from the use of barrier technology. Examples, including unidirectional airflow devices and isolators, of such measures were considered in Chapter 16.

Examination of soil samples using surface per agar counting procedures generally reveals total viable counts in the order of $10^{12}$ fungi and $10^{14}$ bacterial cells per gram. Of these, at least a hundred different bacterial and fungal species will be represented. Air and wind blowing from outside a manufacturing environment will therefore carry with it many bacterial exospores and endospores. These will also impregnate normal outdoor clothing and will be carried on the soles of shoes. This reinforces the need for correct cleanroom entry requirements, basic hand hygiene, and appropriate gowning procedures [7]. Furthermore, in order meet microbial control requirements, all the surfaces within the cleanroom, as well as personnel hands need to be disinfected routinely using a variety of disinfectants [8]. Due to theoretical disinfectant resistance, disinfectants used for the microbial treatment of surfaces are typically rotated and have different modes of action. Normally this means the use of a sporicidal and nonsporicidal agent [9].

Total viable counts for water samples typically vary from between 10 and $10^6$ per milliliter. Generally the higher counts are obtained from water that has remained static such as in stagnant pools, drains and holding tanks, whereas the lower counts are associated with free-running water such as in rivers and streams where the majority of organisms are found fixed to large particles in the riverbed. Within the pharmaceutical environment, water is treated to render it low in microbial contamination [10]. This is through processes like reverse osmosis and distillation [11] (as outlined in Chapter 10). The microorganisms isolated from pharmaceutical water systems are overwhelmingly Gram-negative [12]. Risks arise from poorly designed or maintained water systems; or in situations where stagnant water is allowed to remain for long periods on the floor.

The practical setting of the cleanroom, microbial challenges to pharmaceutical products can be airborne or waterborne. Airborne organisms might vary in concentration from 10 to $10^4$ per liter, depending upon the grade of the cleanroom, and range in size from 1 to 50 μm diameter [13]. Invariably such organisms are carried on rafts of larger particles, like skin detritus. The surfaces of these organisms will generally be dry and bear net negative electrostatic charges. Size and charge may be used to electrostatically remove or physically filter organisms from air supplies. Water-borne organisms can be present in much higher concentrations than airborne cells (around $10^6$/mL). They bear a net negative surface charge and range in size from 1 to 10 μm diameter. Since these cells are killed by desiccation, and then every effort should be made to keep manufacturing environments dry. This is the overriding factor to ensuring good environmental control within the pharmaceutical or healthcare environment.

## 22.4 Sources of microbial contamination

Microorganisms may ingress into pharmaceutical products along with the raw materials used in their manufacture, together with components used for packaging. Such microorganisms might originate from the manufacturing environment (such as from the industrial plant, air, surfaces, and personnel), or they might enter during storage of the product if the packaging is inadequate or faulty. The final, and arguably most severe, microbial challenge to a pharmaceutical product is administered at the hands of the consumer or healthcare provider. Some of these sources are considered below.

### *22.4.1 Raw materials*

Materials of synthetic or semisynthetic origin are, if stored correctly, generally relatively free of microorganisms. Products of natural origin, extracted from or made of animal tissues (including enzymes, growth factors, gelatine, lanolin, etc.), and plant materials (such as gums, leaf, and grain and root/tuber starches) will carry with them many of the microorganisms normally associated with the living plant or animal. Minerals that are mined and extracted will often be contaminated with organisms from the soil. Since these will include bacterial endospores, and then they will often survive the heat treatments involved in subsequent processing. It is inevitable that some of these contaminants may be potentially hazardous to a product, and they might represent an infection risk to the user. Generally, quality control procedures will determine the numbers and types of organisms that contaminate each and every batch of such raw materials.

A satisfactory batch of a raw material prepared to a pharmaceutical grade should be free from specified pathogenic bacteria, and the total bacterial and fungal population will be below a predetermined value appropriate to the particular product. This is assessed through microbial limits testing, as outlined in Chapter 8. It must be appreciated, all the same, that given suitable conditions bacteria might be able to grow, and multiply in such raw materials. It is, therefore, imperative that the raw materials are well packaged and that suitable storage conditions are maintained and shelf lives strictly adhered to. One of the most significant risks arises from the raw material becoming wet.

### *22.4.2 Water*

Water is a major raw material used not only in the manufacture of pharmaceutical products but also in the cleaning of the manufacturing environment and production machinery. In addition, it is a primary requirement for the growth of microorganisms. Contaminated water probably represents the single-most important source of microorganisms that contaminate products. Gram-negative bacteria, such as *Pseudomonas aeruginosa*, are capable of rapid growth in stored water, even if this is apparently free of available nutrients (such as distilled water). In this respect deionized water often contains high numbers of bacteria which have multiplied within the exchange resins

(around $10^4$/mL). Even with distilled water (such as water-for-injection), if left for any time at room temperature, then high populations of bacteria can develop (approximately $10^5$/mL within 24 h). Water used for the cleaning of floors and plant, or stagnant within the drainage units, "u"-bends, etc., and wet areas within and around taps and sinks are particularly prone to microbial contamination and may act as sources of contaminants.

To avoid some of these contamination scenarios, machinery and plant should be thoroughly cleaned, and excess water removed. Residual water in a mixing vessel, if left, will grossly contaminate the next batch of product. Where possible water used in the production areas should be freshly distilled and presterilized. Stored, piped water should be maintained at a temperature which does not permit microbial growth (circulated and heated), and pipework should not contain dead-legs, traps, or ill-fitting joints where microbes can attach and grow. Moreover, in the production environment, surfaces should be kept dry wherever possible. Since those organisms that grow preferentially in water tend to be Gram-negative, they do not readily survive desiccation.

### 22.4.3 Manufacturing environment

#### 22.4.3.1 Air

Microbes present within the air of manufacturing environments may ingress into the products where these are open and exposed. Microorganisms will be associated with dust particles drawn in from outside atmospheres, or they may be generated from the shedding of skin-scales by operators and personnel entering the manufacturing facility.

Aerosols created by the turbulent flow of liquids may also give rise to significant numbers of airborne organisms. Thus, the operation of sinks and drains, the running of water taps, and the vigorous cleaning of plant will, at these times, generate airborne organisms.

Ingress of airborne contaminants from the exterior is generally controlled by containing the production environment and providing it with a separate supply of filtered air. Given that the air is sufficiently rapidly recycled within a well-designed cleanroom, and that separate supplies of air protect the filling and mixing equipment, then it is the bacteria introduced along with the clothing and bodies of personnel that require control. Quality standards for controlled environments relate to the air quality (particulate count) of the room and establish working limits on the numbers of microorganisms collected by settle plates and active air samplers.

#### 22.4.3.2 Equipment and facilities

If equipment is inadequately cleaned between uses, or if it is allowed to collect moisture in ports and recesses which directly come into contact with the product, then such processing items can represent potent, and chronic, sources of contamination. Equipment, and pipelines, should be regularly cleaned and stored in a dry state. Appropriate disinfection of exposed surfaces should be employed where appropriate.

If equipment is to be installed or taken into a controlled area, then steps must be taken to decontaminate it or sterilize it before entry. Such procedures would apply equally to maintenance and cleaning equipment used within the area. Withal, walls, and floors within the production environment will accumulate microorganisms and must be subject to regular cleaning and disinfection in conjunction with microbiological monitoring of the facility [14].

### 22.4.3.3 Personnel

The biggest single source of microorganisms in an enclosed environment arises from the people working within it. Individuals shed many thousands of skin scales to the atmosphere per day. Many of these will be contaminated with skin microorganisms such as yeast-like fungi and bacteria of the genera *Staphylococci* and *Micrococci*. Gowning procedures and hair coverings are intended to minimize such shedding to the environment, and the wearing of facemasks contains aerosols generated by breathing and sneezing.

The axillae of the body and the hair-covered surfaces (armpits, crotch, head, and neck) are ecologically rich niches, and they are especially prone to shedding. Particular attention is, therefore, paid to these areas in the design of specialized clothing for use in the cleanroom and controlled areas. Overshoes prevent the movement of dust borne microorganisms from one area to another. Strict entry procedures must be adhered to, involving hand wash routines, and removal of outside clothing. Personnel must be trained in the use of controlled environments in order to minimize their contribution to airborne contamination. A major objective of such training is to minimize the body movements made by personnel since it is well documented that rapid movements and exertion increase the rate of skin scale shedding and reduce the effectiveness of the protective clothing.

### 22.4.3.4 Users and healthcare professionals

The final abuse that product has to withstand is that given to it by the consumer or healthcare professional for administration to a patient. If the product is multiuse (i.e., opened, used several times, and stored between uses), then microorganisms may enter it at each use. Such organisms may grow during subsequent storage of the opened product. Furthermore, while storage conditions prior to sale can be adequately controlled those at the point of use cannot be. Products might, therefore, be stored in hot humid environments (bathrooms) where microbial growth will be favored. With multiuse products, the risk is elevated.

Contamination by the user is particularly problematic for creams and ointments that are applied by the fingers and hands. Even with products such as eye drops, it is possible for the user to contaminate the applicator and return organisms to the bottle. In some instances (such as modern mascara dispensers and eye drop bottles), the design of the container is intended to minimize consumer contamination. In other instances, this is not possible. Some products contain preservatives, but the preservative cannot deal with all instances of poor practice.

## 22.5 Fate of microbial contamination in pharmaceutical products

Once microorganisms have ingressed into a pharmaceutical or cosmetic product, the contaminating microorganisms will either die immediately (especially if the product is then subjected to a terminal sterilization process), survive for some time but be unable to grow and divide (static contamination) or given conditions favorable for growth, multiply (dynamic contamination).

### *22.5.1 Death of contaminants*

If the contaminating microorganisms that ingress into the product die, then the risks to the consumer often die with them. If, however, the microorganisms had produced toxic materials within the product, either before or as a result of death, then these hazards will remain. Good examples of this phenomenon are pyrogens (especially endotoxin, as discussed in Chapter 11).

Other examples of toxic material, not covered elsewhere in this book, are exotoxins and enterotoxins. Exotoxins are secreted by bacteria or released through cell lysis. Exotoxins can trigger toxic shock-like syndrome (TSLS), characterized by hypotension or shock, fever, and multiorgan system involvement. Prominent examples include botulinum toxin (from *Clostridium botulinum*) and texanospasmin (from *Clostridium tetani*) Among the best-characterized exotoxin are superantigens. These are associated with the family of pyrogenic exotoxins produced by *Staphylococcus aureus* and *Streptococcus pyogenes*. The related routes of infection are primarily food poisoning (via ingestion). Although exotoxins have a degree of heat resistance, this is lower than endotoxin. Therefore, in relation to the manufacture of sterile pharmaceuticals, any risks to contaminated glassware intended to be sterile from Gram-positive microorganisms are eliminated through depyrogenation cycles for glass vials [15].

Another bacterial source comes from enterotoxins, such as from *S. aureus*. Enterotoxins are single-chain globular proteins of varying molecular weights. They are released by bacteria and tend to, as the name implies, target mammalian intestines. It is not clear the level of enterotoxins needed to elicit a pyrogenic response, although the levels are again estimated to be considerably higher than endotoxin (at 1 µg/kg) [16]. Monitoring for high levels of staphylococcal bioburden (especially *S. aureus*) would act as a risk control in pharmaceutical manufacturing.

Other cell-related material that can be pyrogenic is peptidoglycan, common to both Gram-negative and Gram-positive bacteria (although it is found in much higher quantities with Gram-positive bacteria). Peptidoglycan is a bag-shaped macromolecule that surrounds the cell. Although peptidoglycan is demonstrably pyrogenic, very high numbers of Gram-positive bacteria are required to trigger a pyrogenic response (at around $10^8$ cells; in contrast, with endotoxin, a single *Escherichia coli* cell contains about 2 million lipopolysaccharide molecules per cell and as few as 100 cells could trigger a cytokine reaction in humans) [17].

Death of the contaminating organisms will occur if the products are subjected to a sterilization step such as heating in an autoclave. Death will otherwise occur if the prevailing physicochemical environment is unsuitable for growth (pH, water activity, and so forth), or if there are no appropriate nutrients available.

Changing the solute concentration in the medium also changes the osmotic pressure or the water activity $a_w$. Thus, water activity is the vapor pressure of a substance divided by the standard state partial vapor pressure of water at a given temperature. This is expressed as

$$a_w = p/po$$

where $p$ and $po$ are the water pressures of the medium and of pure water, respectively, in isothermal and isobaric conditions.

The water activity is described by Raoult's law, so that

$$a_w = f\left[n_w / \left(n_w + n_s\right)\right]$$

where $n_w$ and $n_s$ are the concentrations of water and solute, respectively. It follows that by increasing the solute concentration, the $a_w$ of the environment decreases. As $a_w$ decreases, the thermal resistance of microorganisms increases [18]. This is why freeze-drying is adopted as preservation methods for several types of foods and pharmaceuticals.

If the microorganisms cannot produce dormant-resting states, such as endospores or exospores, then they will die gradually as the intracellular reserves of nutrients are consumed (starvation-induced death). Death in such circumstances can be slow with individual cells surviving in the product for many months after manufacture. The inclusion of chemical antimicrobial agents within the products as preservatives will not only inhibit the growth of most organisms but may also kill some of the static contaminants.

An endospore is any spore that is produced within an organism usually a Firmicute bacterium (here, the classic examples are *Bacillus* species and *Clostridium* species). This is in contrast to exospores, which are produced by growth or budding. Exospore can also refer to the outermost layer of spore in some algae and fungi. Spores can survive under the most extreme conditions for very long periods of time (in theory, indefinitely). Although spores are non-reproductive, once they are removed from environmental stress and nutrient conditions are appropriate, spores are capable of transformation back to vegetative cells, which are capable of reproduction.

### 22.5.2 Pyrogens and other products of bacterial growth

Gram-negative bacteria release the lipopolysaccharide components of their cell walls into their environment as they grow and divide. Such materials are pyrogenic and are released if the cells are killed. In this instance, release is through degradation of the cells. The amounts of endotoxin released will be in proportion to the numbers of bacteria killed.

The bacterial pyrogens are stable to heating in an autoclave and will if injected into the body of an animal lead to a marked rise in body temperature. Pyrogens must therefore be absent from all injectable products. They can be destroyed by prolonged exposure to high temperatures in a hot-air oven. Terminally sterilized injectable products must, therefore, not contain pyrogens, or high numbers of microorganisms that might liberate them, at the time of autoclaving. Similarly, glassware used for such products must be washed in pyrogen-free water or be depyrogenated, by heating at around 250 °C for 30 min (or greater), prior to use [19]. Other bacterial exoproduct toxins may be allergenic when applied to skin and mucus membranes.

### 22.5.3 Static contamination

So long as there are viable microorganisms remaining within a product then such organisms have the potential to cause infection in the consumer during product use. If the physicochemical conditions within the product change (such as through the growth of a different organism), then static contaminants may be able to recommence growth. The risks of infection presented by any given organism depend upon:

1. the numbers of bacteria that are likely to be present, and viable, within a single dose (i.e., related to cell number);
2. the route of administration of the product.

Many bacteria are more able to initiate infection more readily and with fewer initial numbers of cells, by the parenteral route than, for example, by ingestion. If sterile products are eliminated from this discussion, then the infection risk is related to the presence of viable opportunistic pathogens in the product. These either will be able to infect by the oral route or will be capable of infecting damaged skin.

### 22.5.4 Dynamic contamination

A pharmaceutical product is dynamically contaminated when microorganisms present within it are actively growing and multiplying. This will occur when the physicochemical environment is suitable for growth and when adequate supplies of nutrient are present within the product. Some products will be more liable to promote growth than others.

### 22.5.5 Physicochemical environment

Individual species of bacteria and fungi are capable of growth only within fairly narrow ranges of pH, water activity, and temperature. Within slightly broader ranges of value, growth is inhibited, and the cells survive (so long as sufficient nutrients are available for their subsistence). For obligate pathogens adapted to growth in the human body, the ranges of suitable growth condition are particularly narrow and reflect the homeostatic mechanisms at play in higher animals and plants.

Environmental isolates and opportunistic pathogens are generally able to tolerate much wider ranges of physicochemical conditions. The extreme examples of

microorganisms are unlikely to be capable of initiating infection and are equally unlikely to contaminate pharmaceutical and cosmetic products. The most likely dynamic contaminants of products are mesophilic organisms growing naturally in the manufacturing facility and in the water supplies. While endospores and exospores will contaminate products and are generally resistant to heat and chemical agents they are incapable of multiplying as such. Multiplication involves a breaking of dormancy, germination, and successful growth of the vegetative cell before new endospores or exospores can be formed. Thus, spores might be present as static contaminants of a product with extreme pH or water activity but cannot lead to dynamic contamination unless conditions in the product are altered.

### 22.5.6 Product pH

The pH of a product material is an important factor. Generally, fungi are able to survive and grow at more acid pH (pH 4–6) than the bacteria (pH 5.5–8). Acidic products that are contaminated will, therefore, tend to show signs of fungal rather than bacterial contamination [20]. Since the growth of many bacteria, particularly anaerobic, fermentative organisms lead to the generation of organic acids, and then a product of neutral pH showing dynamic contamination with bacteria will become progressively more acidic.

Eventually acid production will inhibit the growth of the bacteria and facilitate the germination and growth of fungal spores, if present. Dynamic contamination with one type of organisms can therefore lead, at a later stage, to dynamic contamination by previously static organism.

The latter might have been incapable of growth in the unspoiled product. This is referred to as "dynastic" spoilage. In the same manner, the pattern of bacterial growth and spoilage of a product can represent a sequence of bacterial dynasties. Mixtures of microorganisms are commonly found that can spoil a product where individual isolates cannot.

### 22.5.7 Water activity

Gram-negative bacteria are adapted to low osmolarity environments (high water content) and are, therefore, almost exclusively found growing in water and dilute solutions. Gram-positive bacteria, on the other hand, are by virtue of the strength vested in their cell walls, capable of survival and growth in less dilute systems than Gram-negatives [21]. Most moulds prefer even lower water content environments than bacteria, and some can even generate their own water as a by-product of metabolism. In this manner, dilute pharmaceutical systems (e.g., eye drops, infusions, etc.) will tend to show dynamic contamination with Gram-negative bacteria rather than Gram-positives, yeasts, and fungi. Creams, on the other hand, will, if dynamically contaminated, tend to support the growth of Gram-positive organisms and fungi. Very low water content pharmaceuticals (ointments, tablets) will be relatively safe from dynamic contamination by bacteria but might support the growth of some fungi at the

surface of the product if stored inappropriately. This is analogous to the contamination of jams and other preserves where mould will often grow on the surface of the product where water of condensation has formed during filling and where metabolic-water, generated by the fungi themselves, can accumulate.

### 22.5.8 Nutrients

Microorganisms require certain basic nutrients for growth and maintenance of metabolic functions. The amount and type of nutrients required range widely depending on the microorganism. Microorganisms can derive energy from carbohydrates, alcohols, and amino acids. Most microorganisms will metabolize simple sugars such as glucose. Others can metabolize more complex carbohydrates, such as starch or cellulose, or glycogen found in muscle foods. Some microorganisms can use fats as an energy source.

Amino acids serve as a source of nitrogen and energy and are utilized by most microorganisms. Some microorganisms are able to metabolize peptides and more complex proteins. Other sources of nitrogen include, for example, urea, ammonia, creatinine, and methylamines.

Growth of microorganisms will only occur in a product if appropriate nutrients are provided. In order to grow microbes require sources of nitrogen and carbon.

## 22.6 Consequences for microbial growth

The previous section considered the possibility of microbial growth. This section discusses what may happen when growth occurs.

### 22.6.1 Product stability

Microbial growth will lead to reductions in the pH of the formulation. With nonsterile products, this might affect the color of dyes and could cause acid cracking of some emulsions.

With specific product types, excipients such as surfactants can often provide sources of carbon and nitrogen for bacterial growth. If such materials are degraded then they can no longer stabilize the product. Phase separation of oil and water will occur. The texture of creams will be adversely affected by the growth of microorganisms, particularly fungi. The production of gases such as hydrogen sulfide and methane from fermentative metabolism can affect the smell of the product and also cause the creation of gas pockets.

Organic acids produced through fermentative growth of microorganisms can affect both the smell and the taste of products. Many bacteria produce brightly colored metabolites that can drastically alter the physical appearance of the product. Such changes are of particular concern when the product is a cosmetic.

### 22.6.2 Infection risk

Since the infection risk is related to the numbers of microorganisms, a user is exposed to, then microbial multiplication will increase numbers and hence the degree of risk. This is also associated with the route of administration/use of the product. Spoilage organisms are unlikely to be primary pathogens, but, in high number, environmental contaminants might be able to initiate infection in a compromised patient.

### 22.6.3 Therapeutic effect

If the source of nutrient to the contaminant organisms are active ingredients of the product, then the therapeutic efficacy of the product will be compromised as the ingredient is metabolized. In this fashion certain antibiotics such as the beta-lactams, some steroid agents and many antimicrobial preservatives (such as parabens and phenolics) can act as nutrients for microbial growth.

### 22.6.4 Sterile products

All products intended for parenteral, otic, and ophthalmic administration or which might come into contact with damaged or abraded skin (dressings, creams) or mucosa epithelial tissue (bladder) or internal organs (irrigation fluids) use must be manufactured as sterile. Sterility of pharmaceutical products is defined as the total absence in an object or field of all viable forms or life. This is an absolute term, an object or product being either sterile or nonsterile. In conjunction, sterilization is a process that is intended to remove or kill all viable microorganisms from an object or field. Pharmaceutical products may either be subjected to a terminal sterilization process or the component parts may be sterilized separately and assembled into the product and filled into the final containers under aseptic conditions. Aseptic processing is applied to products that are damaged by terminal sterilization processing.

## 22.7 Microbiological testing

This book has emphasized that the best way to ensure microbial control is through product, process, and environmental controls. It remains that, once the microbiologist has had input into these processes, testing must take place to verify that the product is of acceptable microbial quality.

### 22.7.1 Nonsterile products

Microbiological tests on the quality of nonsterile pharmaceuticals are limited to estimates of the total viable numbers of bacteria and fungi, together with tests of preservative effectiveness. The latter is performed as a biological challenge test. The fate of known numbers and types of bacteria and fungi inoculated into the product is followed

over several weeks. There are no regulatory limits for the numbers of viable organisms that can contaminate a nonsterile product provided that it is free of known pathogens. Most companies, however, set limits of <100 organisms/mL [22].

### 22.7.2 Sterile products

The microbiological standard set for sterile products is that they should not contain any viable forms of life. This is an absolute term, products are either sterile or nonsterile. "The sterility test" attempts to demonstrate whether or not a particular item is sterile. The basis of the test is that the contents of a product item added to a nutritious medium that will support the growth of any microorganisms within it. Details of the sterility test were presented in Chapter 12. In addition, sterile products must be apyrogenic and free from visible particles.

## 22.8 Conclusion

Microbiological matters continue to exercise considerable influence on product quality. This chapter pulled together some of the different themes from the book in order to provide an overview of the risks posed from microbial contamination of sterile and nonsterile products. The chapter has also considered some risk mitigation steps, from cleanrooms to antimicrobials; these can either prevent contamination ingress or provide a degree of protection. When assessing such matters, the pharmaceutical microbiologist needs to take a holistic approach.

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