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TECHNOLOGY/APPLICATION

Microbial Diversity in Pharmaceutical Product Recalls and Environments

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ABSTRACT: Identification of microbial contaminants in product recalls and environmental samples provides important information on the possible contamination sources and distribution of microbial species in pharmaceutical environments. Analysis of FDA product recall data for 134 non-sterile pharmaceutical products from 1998 to September 2006 demonstrated that 48% of recalls were due to contamination by either *Burkholderia cepacia*, *Pseudomonas* spp., or *Ralstonia picketti,* while yeast and mold contamination were found in 23% of recalls. Gram-negative bacteria accounted for 60% of recalls, but only 4% were associated with Gram-positive bacteria. Of the 193 recalls of sterile products, 78% were due to the lack of sterility assurance and 7% for yeast and mold contamination. For sterile products, Gram-negative bacteria accounted for 6% of recalls, with only 1% due to Gram-positive bacteria. For non-sterile and sterile products, *B. cepacia* was the most frequently isolated microbial species with 22% and 2.5% of recalls, respectively. Based upon the review of the scientific literature, *B. cepacia*, *Pseudomonas* spp., or *Ralstonia picketti* may be associated with water contamination, while yeast and mold and Gram-positive bacteria may have indicated deficient environmental controls. The presence of unculturable microbial populations in pharmaceutical waters and clean rooms was reported, but no evidence has been published that product quality was negatively affected.

KEYWORDS: Microbial contamination, Recalls, Microbial diversity, Microbial control

Introduction

Microbial contamination control in the pharmaceutical industry is a multidisciplinary approach requiring the interaction of microbiology, engineering, and chemistry (1–3). Because microorganisms are ubiquitous to the environment, during construction of pharmaceutical facilities systems are developed and validated to contain and control their numbers, distribution, and growth (2). Microbial distribution and growth in pharmaceutical environments is limited by environmental gradients. Microorganisms survive and grow within different gradients in temperature, available water, pH, organic compounds concentration, and in other factors (4). These gradients have thresholds that are the limits above and below microorganisms cease to function and die. Optimization of microbial contamination control requires the development and implementation of systems leading to environmental fluctuations that will minimize or eliminate microbial survival and growth (1, 2). However, the presence of objectionable microorganisms in non-sterile products—or any type of microorganism in sterile products—indicates lack of process control and system optimization. Identification of microbial contaminants provides important information to track contamination sources, implement proper corrective actions, and understand microbial community composition (4, 5). As part of environmental monitoring and quality control testing of finished products and raw materials, microbial identification of environmental isolates and contaminants is based upon the criticality of a given situation (5). Some of the situations stated by Cundell include media fills, water excursions, product contamination, exceeding microbial environmental levels for personnel, surfaces, and air (5).

Friedman (6) recently discussed several contamination incidents to illustrate the impact of lack of process control on sterile products quality. The purpose of this article is to further that discussion by reviewing Food and Drug Administration (FDA) recalls due to microbial contamination for non-sterile and sterile pharmaceutical products from 1998 to 2006 and relevant studies on microbial diversity in pharmaceutical envi-

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ronments, to discuss the types of microbial contaminants and their possible sources.

Microbial Diversity in Product Recalls (Non-Sterile Pharmaceutical Products)

What types of microorganisms have been reported in product recalls? That information can be very important to understand the sources of microbial contamination. Microorganisms do not have to grow to spoil pharmaceutical products because the catabolic products of microorganisms can break down a given formulation (7). Furthermore, the presence of high numbers of microorganisms and pathogens represents a serious health threat to consumers, as products will be ingested or applied to human skin. On the basis of FDA recall data from 1998 to September 27, 2006, heterotrophic microorganisms caused the majority of microbial contamination reported in non-sterile pharmaceutical products. A summary of microbial contaminants for non-sterile pharmaceutical products in the US is shown in Table I. Some of the products are liquids, tablets, capsules, oils, drops, creams, and emulsions. The pH of the recalled formulations ranged from acidic to alkaline. Evidently, microorganisms are capable of contaminating a given pharmaceutical formulation regardless of water content, pH, or manufacturing process.

Of the 134 recalls reported by the FDA, 60% were associated with contamination by Gram-negative bacteria, while Gram-positive bacteria were found in only 4% of recalls. The numbers suggest that Gram-negative bacterial contamination appeared to be a more serious problem that Gram-positive bacteria. Gram-negative contamination might have come predominantly from water and raw materials. When analyzing the different types of microbial species isolated from recall samples, *Pseudomonas* spp., *B. cepacia*, and *R. pickettii* contamination accounted for 48% (Table I) (4, 9–12). These types of bacterial species are waterborne contaminants that cause major problems in water systems when sanitization and operation are deficient. Water is known to be the most common raw material in pharmaceutical manufacturing. Water is also used to rinse and clean equipment, floors, and walls. Drinking water is physically and chemically treated to reduce microbial numbers and pathogenic microorganisms, but water for pharmaceutical processes is further treated to minimize microbial numbers, endotoxin substances, and organic and inorganic compounds (1–3). The fewer organic compounds in water, the fewer microorganisms that will be found because microbes use organic compounds to survive and grow. Bacterial species such as *Pseudomonas* spp., *Alcaligenes* spp., *Stenotrophomonas* spp., *Burkholderia cepacia*, *Ralstonia picketti*, *Serratia* spp., and *Flavobacterium* spp. were commonly found in water samples (13–18). Other species reported were *Pseudomonas aeruginosa, Pseudomonas fluorescens, Pseudomonas alcaligenes, Flavobacterium aureum, Acinetobacter lowffi,* and *Brevundimonas diminuta*. All these bacterial species can be considered facultative oligotrophs because they are capable of slow growth under low concentrations of organic compounds. When growing under those conditions bacteria reduce cell size and surface-volume ratio, and they undergo some metabolic changes. Bacterial species discussed above are capable of biofilm formation in water distribution systems that can cause serious problems if proper sanitization and maintenance procedures are not followed (1, 2).

Recent studies expanded our understanding of the microbial communities in pharmaceutical waters by using 16S ribosomal analysis, direct DNA extraction, polymerase chain reaction (PCR) amplification, and denaturing gradient gel electrophoresis (DGGE) testing. Results demonstrated the presence of the following culturable bacterial species: *Bradyrhizobium* spp., *Xanthomonas* spp., *Stenotrophomonas* spp., *Methylobacterium spp.*, and *Aquaspirillum* spp. (14, 19). However, the predominant bacterial type in the water system could not be detected on culture media such as soybean casein digest agar (SCDA) or R2A. 16S rDNA sequencing analysis identified the non-culturable species to be members of the *Alphaproteobacteria* with no close relationship with previous culturable or identified species. Non-culturable species of *Mycobacterium* spp. were also detected using PCR analysis (15). Previous studies using epifluorescence microscopy and viability dyes detected a large portion of active bacteria unable to grow on R2A and SCDA media (17). For instance, microbial numbers using epifluorescence microscopy with viability dyes and flow cytometry analyses were 2 times higher than regular plate counts (15, 17, 18).

Molds and yeasts were also common contaminants in non-sterile product recalls, although not generally speciated (or at least not reported by species) (Table I). Contamination by yeast and mold was found to be the second cause for product recall. Twenty-three percent of recalls were due to yeast and mold contamination.

TABLE I
FDA Recalls of Non-Sterile Products from 1995–2006 (8–12)

Product	Reason
Acetaminophen	aerobic microorganism
Aminocaproic syrup	yeast
Benzyl peroxide solution	*Burkholderia cepacia*
Topical cream	*Pseudomonas putida*
Triclosan lotion	*Pseudomonas aeruginosa*
Acne cream	*Burkholderia cepacia*
Albuterol sulfate inhalation solution	*Burkholderia cepacia*
Albuterol sulfate syrup	*Burkholderia cepacia*
Barium sulfate	mold
Ursodiol Cap	potential microbial contamination
Vera Gel	*Enterobacter gergoviae*
Non-alcohol body spray	*Burkholderia cepacia*
Triple S gentle wash	*Pseudomonas aeruginosa*
Amicar syrup	*Candida parapsilosis*
Sodium chloride cleanser	*Pseudomonas aeruginosa*
Albumin human 5%	*Enterobacter cloacae*
Eye gel	*Pseudomonas aeruginosa*
Mouth rinse anti plaque alcohol-free	*Burkholderia cepacia*
Medical food nutrition supplement	*Pseudomonas aeruginosa*
Dialysate concentrate	bacterial contamination
Tylenol gelcaps	aerobic microorganisms
Brand baby oil	*Burkholderia cepacia*
Wet and wild liquid makeup	*Pseudomonas aeruginosa*
Topical product	*Pseudomonas aeruginosa*
Dial brand dialyte concentrate	mold
F12 nutrient mixture	bacterial contamination
Gelusil liquid anti-gas antacid	*Bacillus* spp.
Hydrox alcohol-free mouthwash	*Burkholderia cepacia*
Electrolyte solution	*Aspergillus niger*
Dry skin creme	mold
Neoloid emulsified castor oil	exceeds microbial limits
Mouth rinse alcohol-free	*Burkholderia cepacia*
Fresh Breath Plus mouthwash	*Pseudomonas aeruginosa*
Fresh Moment alcohol-free mouthwash	*Burkholderia cepacia*
Children's cologne	*Pseudomonas aeruginosa*
Vinegar and water douche	mold
Skin creme	mold
Preparation H ointment	mold
Penecare lotion	*Candida lipolytica*
Aidex spray cleaner	mold
Mouth rinse antiplaque alcohol-free Oral B	*Burkholderia cepacia*
Aloe vera cream	*Burkholderia cepacia*
Antacid-antigas liquid suspension	bacterial contamination
Sea therapy mineral gel	*Pseudomonas aeruginosa*
	Pseudomonas fluorescens

TABLE I
(continued)

Product	Reason
Shampoo exotic fruits	bacterial contamination
Mouthwash alcohol-free	*Pseudomonas aeruginosa*
Medical food nutrition supplement	*Pseudomonas aeruginosa*
Panama jack tanning lotion	bacterial contamination
Acne treatment cream	*Burkholderia cepacia*
Astringent pad	mold
Oral suspension	yeast
Clinical resource food supplement	*Pseudomonas aeruginosa*
Nystatin oral suspension	possible microbial contamination
Kenwood brand emulsified castor oil	exceeds microbial limits
Fluoride mouthrinse	*Burkholderia cepacia*
Benzoyl peroxide wash	potential for microbial contamination
Shampoo (anti-dandruff)	*Burkholderia cepacia*
Misoprostal tablets	*Burkholderia cepacia*
Simethicone drops	*Burkholderia cepacia*
Vitamin E–lanolin lotion	mold
Nutritional beverage powders	may contain *Salmonella* spp.
Formance	may contain *Salmonella* spp.
Hand and body lotion with lanolin	mold
Cytotec tablets	*Pseudomonas* spp.
Propac protein supplement	may contain *Salmonella* spp.
Sodium fluoride oral mouth	mold
Soylac infant formula	may contain *Salmonella* spp.
Ben-Agua wash	potential for contamination
HEB cream base	mold
Kayolin pectin suspension	microbial contamination
Antacid oral liquid suspension	bacterial contamination
Body wash and shampoo	*Klebsiella oxytoca*
Hygienic wipe pads	molds
Eye shadow	*Pseudomonas stutzeri*
Soy protein infant formula	*Klebsiella pneumoniae*
	Pseudomonas aeruginosa
Cream base	mold
Oral suspensions	yeast
Antacid-antigas oral	bacterial contamination
Aloe skin cream	*Burkholderia cepacia*
Food industry sanitizing soap	*Burkholderia cepacia*
Hand disinfectant and body lotion	*Burkholderia cepacia*
Shampoo	*Burkholderia cepacia*
Alcohol free mouthwash	*Pseudomonas aeruginosa*
Cough syrup	exceeds microbial limits
Disinfectant first aid treatment	*Burkholderia cepacia*
Sunburn gel and spray	*Burkholderia cepacia*
Anti-plaque alcohol-free mouth rinse	*Burkholderia cepacia*

TABLE I
(continued)

Product	Reason
Infant formula	non-pathogenic spoilage
	microorganisms
Boric acid solution	exceeds microbial limits
Minocycline capsules	microbial contamination
Myla-care antacid anti-gas liquid	bacterial contamination
Sodium chloride	*Ralstonia pickettii*
Benzalkonium chloride towelette	*Burkholderia cepacia*
Calcitriol	*Bacillus cereus*
Syrup	*Staphylococcus warneri*
Haloperidol oral solution	microbial contamination
Hydrocortisone polistirex suspension	microbial contamination
Lidocaine HCl/epinephrine injection	microbial contamination
Colostrum cream	*Pseudomonas putida*
Eye and ear drops	*Pseudomonas fluorescens*
Opthalmic solution	*Burkholderia cepacia*
Antiseptic solution	*Pseudomonas aeruginosa*
Nystatin oral suspension	*Acinetobacter baumanii*
Povidone-iodine solution	*Pseudomonas putida, Salmonella* spp.
	Aeromonas sobria
Bactroban ointment	*Ralstonia pickettii*
	Pseudomonas fluorescens
Gel	microbial contamination
Bicarbonate concentrate	mold contamination
Simethicone solution	microbial contamination
Ampicillin suspension	mold contamination
Anthacid liquid	*Bacillus licheniformis*
Eye and nasal drops	*Pseudomonas mendocina*
	Klebsiella pneumoniae
Progesterone cream	mold contamination
Mouthwash	*Pseudomonas alcaligenes*
	Pseudomonas baleurica
Nasal spray	*P. fluorescens*
Antacid liquids	*Enterobacrer cloacae*
	Citrobacter freundii
	Klebsiella pneumoniae
	Flavimonas oryzihabitans
	Salmonella arizonae
Oleic acid	yeast
Laxative solution	mold
Acetaminophen tablets	mold
Medicated hand wash	*Pseudomona spinosa*
Antiseptic mouthwash	yeast and mold contamination
Nasal spray	*B. cepacia*
Hand sanitizers	bacterial contamination

TABLE I
(continued)

Product	Reason
Nasal spray	*B. cepacia*
Oral pharmaceuticals	mold, yeast
Calcium carbonate, simethicone solution	*S. aureus*
Tablets	mold
Tablets	mold
Pharmaceutical topical creams	microbial contamination
Oral pharmaceuticals	microbial contamination
Antibacterial hand soap	*P. aeruginosa*
Gel capsules	*P. aeruginosa*
Tablets	mold
Oral pharmaceuticals	*P. aeruginosa, B. cepacia*
Dimethicone solution	*B. cepacia*

Manufacturing of non-sterile pharmaceuticals does not typically follow the same type of environmental control as sterile products. Air flowing into the facility is not filtered through 0.5-micron filters. For instance, environmental parameters such as temperature, humidity, and pressure are not controlled as rigorously as in clean rooms. Therefore, yeast and mold contamination might have been airborne contaminants. Movement of materials, equipment, and personnel through manufacturing areas is not restricted, therefore they may also contribute to mold contamination. To develop a more strict control of non-sterile manufacturing, some companies are moving into loosely following environmental conditions such as the ones described for class 100,000 clean rooms. Some of the microbial species commonly found in air samples are bacteria such as *Bacillus* spp., *Staphylococcus* spp., and *Corynebacterium* spp. Commonly mold species are *Aspergillus* spp., and *Penicillium* spp. (4). Because of the less stringent environmental controls during production, microbial diversity in non-sterile pharmaceutical facilities is higher than in controlled environments used for sterile manufacturing.

Of the USP objectionable bacteria, the percentages of samples showing the presence of *P. aeruginosa*, *Salmonella* spp., and *S. aureus* were 13%, 5%, and less than 1%, respectively. None of the recalls indicated the presence of *E. coli* (Table I). When looking at the different microbial species reported, the most frequently found microbial species were *B. cepacia,* with 22% of recalls reported. At this time none of the pharmacopeias recommends *B. cepacia* to be one of the objectionable microorganisms. However, analysis of recall data supports the inclusion of *B. cepacia* as one of the objectionable microorganisms for microbial limit test of non-sterile pharmaceuticals. *B. cepacia* is a nutritionally versatile, widespread Gram-negative bacterium that based upon recall data is as relevant to pharmaceutical quality control as current indicators. The original objectionable bacterial list was based upon the pathogenicity of *E. coli*, *P. aeruginosa*, *S. aureus*, and *Salmonella* spp. (20). Expanding the objectionable list with *B. cepacia* will provide an indicator for process control optimization. *B. cepacia* is also a common nosocomial pathogen, with numerous incidents of morbidity associated to contaminated drugs (21, 22). Numerous reports from the 1980s and early 1990s indicated that contamination by *B. cepacia* was becoming a major problem in pharmaceutical products (23, 24). However, up to this point no systematic analysis of recalls was performed over time to determine the importance of this microbial contaminant. The data discussed confirm the negative impact of *B. cepacia* contamination on the quality control of non-sterile pharmaceutical products.

Raw Materials as Possible Sources of Microbial Contamination for Non-Sterile Products

Raw materials and excipients utilized for the development of non-sterile formulations are based upon natural products, which contain a high microbial load. Testing must be performed to determine the quality of these materials. Absence of objectionable microorganisms is required before raw materials are used in

non-sterile pharmaceutical products (8). However, other types of microorganisms can also be hazardous. Cundell (24) discussed several alternatives to manage the microbiological quality of excipients. He stated that environmental conditions during manufacturing and storage of excipients led to significant contamination incidents. Some of the conditions described were high humidity during storage of raw materials, which increased the amount of water available for microbial growth, and failure to clean and sanitize equipment, leading to higher organic carbon concentration (24). Furthermore, some raw materials come from natural sources, which can be animal, plant, or mineral, while others are classified as synthetic or semi-synthetic. Chemicals from natural sources exhibit a more diverse and abundant microbial load than synthetic and semi-synthetic sources because manufacturing processes for the latter do reduce microorganisms (24). Furthermore, some product manufacturing processes are designed to significantly reduce the numbers of microorganisms. Different types of bacteria commonly found in pharmaceutical raw materials were *Brevibacter* spp., *Proteus* spp., *Enterobacter* spp., *Klebsiella* spp., *Serratia* spp., *Lactobacillus* spp., *Pseudomonas* spp., *Bacillus* spp., *Escherichia* spp., *Streptoccocus* spp., *Clostridium* spp., *Agrobacterium* spp. (25–30). Molds such as *Aspergillus* spp., *Penicillium* spp., *Cladosporium* spp., and *Fusarium* spp. were also reported. Some of the bacterial genera mentioned above other than *Pseudomonas* spp. were implicated in 12% of non-sterile recalls (Table I). Anaerobic bacteria such as *Bifidobacteria* spp. and *Clostridium* spp. were reported only in natural raw materials but were not found to be present in any of the products recalled. In these studies, biochemical and molecular analyses were used to enumerate and characterize the microbial load of active ingredients and excipients (25–30). For instance, PCR analyses were used for quality control testing of raw materials such as gelatin and carboxymethylcellulose and for characterization of microbial contamination (23, 29–32). Specific bacterial and mold sequences were used to accurately screen and identify microbial contaminants in raw materials. Identification by genetic testing complemented biochemical analyses, providing greater resolution and accuracy when biochemical analyses failed to characterize microbial isolates (29, 30).

Microbial Diversity in Product Recalls (Sterile Pharmaceutical Products)

From a total of 197 recalls covering 1998 to September 27, 2006, the lack of sterility assurance appeared to be the number one reason for product recalls (Table II) (9–12, 33). Over the last 8 years, 78% of sterile product recalls were due to lack of sterility assurance. Some of the reasons given were package integrity deficiencies, media-fill failures, improper sterilization validation, and numerous deficiencies during aseptic processing. Lack of sterility assurance is a major good manufacturing practice (GMP) violation—evidently adequate validated and documented processes were not followed. Therefore, as determined by regulatory agencies, the probability and risk of introducing microorganisms into the products were beyond acceptable levels. If a package for a sterile product is not sealed and its integrity is questionable, there is a high probability that microorganisms can get into that package and compromise drug safety and potency. If sterilization validation was not properly performed, there is a high probability that some microbial cells survive and may contaminate the product. If the media fill was not successful and contaminated vials were found, aseptic processing was not properly designed and executed, indicating possible flaws in the manufacturing process. Introducing microorganisms in contaminated parenteral drugs and medical devices can result in morbidity and mortality. Products such as injections and medical devices must be and remain sterile with a high degree of sterility assurance.

Several cases of microbial contamination of sterile products were detected by sterility testing, even though the numbers of samples tested are statistically low when compared to the total number of samples per lot (33). For instance there are 3000 units in a given lot and only 40 are tested, this imposes a tremendous statistical limitation to the test (34). If contamination is detected by sterility test, gross contamination might have probably occurred. However, if a small percentage of product containers are contaminated, sterility testing may not detect the contamination (34). Furthermore, because of the culturable nature of the test, the species of microorganisms detected by sterility testing are affected by the type of media used, incubation temperature, and incubation time (34).

Once samples were found contaminated and isolates were identified, there were different types of microorganisms in contaminated sterile products. Gram-negative bacteria were found in 6% of recalls, while Gram-positive bacteria accounted for only 1%. Gram-negative microorganisms such as *Serratia* spp., *Methylobacterium* spp., *Stenotrophomonas maltophilia*, *Burkholderia cepacia*, and *Ralstonia pickettii*,

TABLE II
FDA Recalls of Sterile Products from 1998–2006 (9–12, 33)

Product	Reason for Recall
Albuterol inhalation solution	*Serratia* species contamination
Baclofen injection	*Penicillium* spp. mold
	Methylobacterium spp.
	Mycobacterium chelonae
Methylprednisolone injection	*Penicillium* spp. mold
	Methylobacterium spp.
	Mycobacterium chelonae
Ceftazidime injection	lack of sterility assurance
Cistracurium injection	lack of sterility assurance
Mivacurium injection	lack of sterility assurance
Doxorubicin injection	lack of sterility assurance
Epirubicin injection	lack of sterility assurance
Fluconazole injection	lack of sterility assurance
Homeopathic eye drops	*Stenotrophomonas maltophilia*
Medroxyprogesterone injection	lack of sterility assurance
Multi-vitamin injection	lack of sterility assurance
Various antibiotic solutions	lack of sterility assurance
Sodium Chloride eye wash	lack of sterility assurance
Succinylcholine injection	lack of sterility assurance
Zidovudine injection	lack of sterility assurance
Various injectables products	lack of sterility assurance
Parenteral product	mold, *Methylobacterium* spp.
	Mycobacterium chelonae
Various injectable products	lack of sterility assurance
Fluconazole injection	lack of sterility assurance
Midazolam injection	lack of sterility assurance
Technetium Tc99m albumin injection	lack of sterility assurance
Vercuronium injection	lack of sterility assurance
Various injectables	lack of sterility assurance
Ophthalmic gel	lack of sterility assurance
Inhalation solution	lack of sterility assurance
Alcohol pads	lack of sterility assurance
Aprotinin injection	lack of sterility assurance
Cefuroxime injection	lack of sterility assurance
Meperidine injection	lack of sterility assurance
Methylprednisolone injection	lack of sterility assurance
Polyvinil alcohol opththalmic injection	lack of sterility assurance
Sodium bicarbonate injection	lack of sterility assurance
Quinupristin/dalfopristin injection	lack of sterility assurance
Saline ophthalmic solution	*B. cepacia* contamination
Heparin injection	lack of sterility assurance
Living skin construct	*B. cepacia* contamination
Serum	bacterial contamination
Medical device	microbial contamination

TABLE II
(continued)

Product	Reason for Recall
Medical device	mold contamination
Medical device	lack of sterility assurance
Medical device	mold contamination
Medical device	mold contamination
Ceftazidine injection	lack of sterility assurance
Ceftazidine injection/cefazolin injection	lack of sterility assurance
Lidocaine HCl/epinephrine injection	lack of sterility assurance
Lidocaine HCl/epinephrine injection	microbial contamination
Oxfloxacin otic solution	lack of sterility assurance
Ticacillin[s1] disodium/clavulanate	lack of sterility assurance
Potassium injection	
Various injectables	microbial contamination
Glycyrrhizinic acid injection	mold contamination
Sodium chloride respiratory therapy	*Ralstonia pickettii*
Injectable solutions	lack of sterility assurance
Medical device	lack of sterility assurance
Methylprednisolone Acetate injection	Mold
Various injectable solutions	lack of sterility assurance
Medical device	microbial contamination
Medical device	microbial contamination
Injectable solutions	lack of sterility assurance
Injectable solutions	lack of sterility assurance
Medical device	lack of sterility assurance
Injectable solution	lack of sterility assurance
Injectable solution	lack of sterility assurance
Injectable solution	lack of sterility assurance
Medical device	mold
Medical device	microbial contamination
Medical device	lack of sterility assurance
Medical device	lack of sterility assurance
Medical device	lack of sterility assurance
Medical device	lack of sterility assurance
Injectable solution	lack of sterility assurance
Injectable solution	lack of sterility assurance
Medical device	lack of sterility assurance
Medical device	lack of sterility assurance
Injectable solutions	*Bacillus licheniformis*
Injectable solutions	lack of sterility assurance
Medical devices	lack of sterility assurance
Medical devices	lack of sterility assurance
Medical device	lack of sterility assurance
Medical device	lack of sterility assurance
Medical device	lack of sterility assurance
Medical device	lack of sterility assurance

TABLE II
(continued)

Product	Reason for Recall
Medical device	lack of sterility assurance
Medical devices	microbial contamination
Medical devices	lack of sterility assurance
Medical devices	lack of sterility assurance
Eyes drops	lack of sterility assurance
Medical devices	lack of sterility assurance
Medical devices	lack of sterility assurance
Medical devices	lack of sterility assurance
Injectable solutions	lack of sterility assurance
Medical device	lack of sterility assurance
Medical devices	lack of sterility assurance
Medical devices	lack of sterility assurance
Eyes drops	microbial contamination
Medical device	lack of sterility assurance
Medical device	lack of sterility assurance
Otic suspension	lack of sterility assurance
Medical device	lack of sterility assurance
Medical device	lack of sterility assurance
Medical device	bacterial contamination (Gram-positive rods)
Medical device	lack of sterility assurance
Medical device	lack of sterility assurance
Sinus relief product	mold and yeast contamination
Medical device	lack of sterility assurance
Medical device	lack of sterility assurance
Medical device	lack of sterility assurance
Medical device	lack of sterility assurance
Medical device	lack of sterility assurance
Medical device	lack of sterility assurance
Injectable solutions	lack of sterility assurance
Medical device	lack of sterility assurance
Medical device	lack of sterility assurance
Medical device	lack of sterility assurance
Medical device	lack of sterility assurance
Medical device	*B. cepacia*
Medical device	lack of sterility assurance
Injectable solutions	lack of sterility assurance
Medical device	lack of sterility assurance
Injectable solutions	lack of sterility assurance
USP purified water	*B. cepacia*
Injectable solution	microbial contamination
USP purified water	*B. cepacia*
Medical device	lack of sterility assurance
Medical device	lack of sterility assurance
Medical device	lack of sterility assurance

TABLE II
(continued)

Product	Reason for Recall
Medical device	lack of sterility assurance
Medical device	lack of sterility assurance
Medical device	lack of sterility assurance
Medical device	lack of sterility assurance
Medical device	lack of sterility assurance
Medical device	lack of sterility assurance
Inhalation solution	lack of sterility assurance
Medical devices	lack of sterility assurance
Medical devices	lack of sterility assurance
Injectable solutions	lack of sterility assurance
Injectable solutions	lack of sterility assurance
Medical devices	lack of sterility assurance
Medical device	*Aspergillus* spp., *Penicillium* spp.
Injectable solutions	lack of sterility assurance
Injectable solution	lack of sterility assurance
Medical device	lack of sterility assurance
Ophthalmic solutions	lack of sterility assurance
Medical devices	lack of sterility assurance
Medical devices	lack of sterility assurance
Medical devices	lack of sterility assurance
Injectable solutions	bacterial contamination
Nasal solution	lack of sterility assurance
Medical device	lack of sterility assurance
Medical device	lack of sterility assurance
Medical device	lack of sterility assurance
Medical device	lack of sterility assurance
Ophthalmic solutions	lack of sterility assurance
Medical device	lack of sterility assurance
Medical device	lack of sterility assurance
Medical device	lack of sterility assurance
Injectable solution	lack of sterility assurance
Medical device	lack of sterility assurance
Medical device	lack of sterility assurance
Medical device	lack of sterility assurance
Medical device	lack of sterility assurance
Medical devices	lack of sterility assurance
Pharmaceutical extract	lack of sterility assurance
Oral inhalation products	lack of sterility assurance
Medical device	lack of sterility assurance
Ophthalmic solutions	bacterial contamination
Medical device	lack of sterility assurance
Medical device	lack of sterility assurance
Medical device	lack of sterility assurance
Medical devices	lack of sterility assurance

TABLE II
(continued)

Product	Reason for Recall
Medical device	microbial contamination
Ophthalmic solutions	lack of sterility assurance
Medical device	negative sporicidal activity
Medical device	lack of sterility assurance
Injectable solutions	lack of sterility assurance
Ophthalmic solution	*P. aeruginosa*
Infusion solutions	microbial contamination
Injectable solutions	bacterial contamination
Nasal solution	lack of sterility assurance
Medical device	lack of sterility assurance
Lubricant eye gel	microbial contamination
Injectable solution	lack of sterility assurance
Lubricant eye gel	lack of sterility assurance
Injectable solutions	lack of sterility assurance
Medical device	microbial contamination
Inhalation solution	lack of sterility assurance
Medical device	microbial contamination
Medical device	lack of sterility assurance
Medical device	lack of sterility assurance
Medical device	lack of sterility assurance
Medical device	lack of sterility assurance
Medical device	lack of sterility assurance
Injectable solution	lack of sterility assurance
Medical device	lack of sterility assurance
Medical device	lack of sterility assurance
Medical device	lack of sterility assurance
Medical device	infections with *Fusarium* spp.

as previously discussed, can indicate problems in the water system by lack of sanitization or incorrect system design (Table II). The most abundant microbial species was *B. cepacia,* with 2.5% recalls. Contamination by the presence of *Mycobacterium* spp. accounted for 2% of recalls. As previously discussed, *Mycobacterium* spp. were associated with water contamination. Yeast and mold contamination was found to be responsible for 7% of recalls. The presence of mold such as *Penicillium* spp. and *Aspergillus* spp. might have indicated improper sanitization of surfaces and lack of controls in air circulation. Products subjected to recall ranged from injectable solutions to medical devices (Table II). Friedman (5) reported that aseptic processing contamination problems were due to one or the combination of three major factors: poor personnel practice, lack of environmental control, and erroneous operational design. Schroeder (35) pointed out that filter failures could also be an important cause for sterility failures during aseptic manufacturing.

Other Possible Sources of Microbial Contamination for Sterile Pharmaceutical Products

The facilities where sterile products are manufactured are basically controlled environments, for example, clean rooms, where people and materials will move in and out to carry out different processes. However, this movement is severely restricted and not as permissive as in non-sterile manufacturing. Furthermore, raw materials used in aseptic manufacturing are mostly free of microorganisms or contain extremely low numbers (36). Filtration and processing during aseptic manufacturing eliminate all microorganisms from finished product samples.

Because microorganisms are commonly associated to particles in the air, the numbers and sizes of particulates in clean rooms are controlled by high efficiency, 0.5-micron high-efficiency particulate air (HEPA) filters (37, 38). Particulates can come from people and processes. Therefore, exclusion of these particles in facilities minimizes the chances of microbial distribution and contamination by air. However, studies demonstrated that the majority of microbial species isolated in clean rooms originated from personnel present in the room during manufacturing (37, 38). This is because microorganisms are normal flora of the human skin and body. Hyde (37) stated that more than 10^{14} living bacterial cells are part of the normal human flora, with 10^{12} from skin, 10^{10} from the mouth, and 10^{14} from intestinal sources. Microorganisms are dispersed from human skin cells. Some of the species living in the human skin are *Staphylococcus epidermidis, S. capitis*, *S. hominis*, *Corynebacterium* spp., *Propionibacterium* spp., *P. acnes*, *Micrococcus* spp., and *Kokuria* spp. Normal flora for the human oral cavity is comprised of *Streptococcus salivarius*, *S. mutans*, *S. sanguis*, and others. Mold can also be a possible contaminant. Common mold from human flora are *Trichophyton* spp., *Epidermophyton* spp., and *Microsporon* spp., and others. Intestinal normal flora belong to species such as *Enterobacter* spp., *Escherichia* spp., *Lactobacillus* spp., *Bifidobacterium* spp., *Bacteroides* spp, and *Clostridium* spp.

To protect critical areas from human microbial flora, personnel wear gowns, hair covers, hoods, shoe covers, laboratory coats, facemasks, gloves, and boots (2). Favero et al. (38) demonstrated that the more controlled the room becomes, the lower the numbers of microorganisms associated with dust and soil such as mold and *Bacillus* spp. It's important to mention that not a single recall incident from 1998 to September 2006 was due to the presence of Gram-positive bacteria originating from bacterial species of human skin normal flora such as *Staphylococcus* spp., *Kokuria* spp., *Corynebacterium* spp., or *Propionibacterium* spp. Only 1% of recalls was due to the presence of Gram-positive rods (Table II). Studies demonstrated that microbial contaminants in class 100 laminar flow hoods were characterized as normal human flora such as *Staphylococcus* spp., *Kokuria* spp., and *Corynebacterium* spp. (38). However, *Bacillus* spp. and mold were isolated most frequently from less controlled environments. As previously described, mold and *Bacillus* spp. are commonly associated with particles of soil and dust. To minimize their presence, continuous sanitization and disinfection of the clean rooms eliminate dust and soil particles, providing a hostile environment for microbial survival and growth.

Controlled environments are provided with humidity, ventilation, and air conditioning units, that control these parameters (2). To exclude any non-viable and viable particle from entering critical areas, airflow and pressure are normally controlled. Humidity also controls the numbers of microorganisms in a room. The more humid the room, the more chances for microorganisms to be carried by droplets of moisture (37). Available water is required for microbial growth. The less water available, the harder is for microorganisms to survive and multiply. Therefore, a dry room provides more hostile conditions for microbes to grow than a humid room does.

Recent studies using 16S ribosomal DNA analysis, direct DNA extraction, and sequencing demonstrated a greater diversity of microorganisms in clean rooms. Some of the species found were *Taxeobacter* spp., *Flexibacter* spp., *Cytophaga* spp., *Ultramicrobacterium* spp., *Stenotrophomonas* spp., *Streptococcus* spp., *Sphingomonas* spp., and *Comamonas* spp. (39). Some of these bacterial species were unable to grow on conventional media. Other studies demonstrated that bacteria suspected to be unculturable in clean rooms were shown to be oligotrophic in nature and were counted and isolated using low-nutrient media after incubation for 28 days (40). Fortunately, all slow-grow bacterial species were as susceptible to disinfectants as the fast-growing types.

Viable but Non-Culturable (VBNC) Bacteria in Pharmaceutical Samples: Do We Have a Problem?

Is it possible that uncultured microbial species reported in clean rooms and water will contaminate finished products? Several studies and industry's daily operations demonstrated the absence of objectionable or any type of microorganisms in pharmaceutical finished products by using compendial methods, adenosine triphosphate (ATP) bioluminescence, PCR analysis, DNA sequencing, and direct viable counts (23, 29, 41–44). These studies illustrated the robustness of pharmaceutical systems working under optimized process control by demonstrating the lack of microbial contamination in all products tested. What is the origin of these unculturable bacteria? Similar results were reported in soil and water samples where environmental fluctuations resulted in the lack of nutrients, with

bacterial cells undergoing starvation conditions (45, 46). When bacterial cells encountered these environmental fluctuations and nutrient depletion, they responded by not growing or growing extremely slowly, leading to microbial metabolism shifting from growth to maintenance (45, 46). These cells have been called viable but not culturable (VBNC) bacteria. Bacteria undergoing this physiological state reduced their metabolism, cell size, and changed their enzymes and protein profiles (45, 46).

Because of the extreme conditions encountered by microbial cells during terminal sterilization of sterile products, it's highly improbable to have any VBNC bacteria in finished product samples. Validation studies for terminal sterilization comprise the use of biological indicators, for example, bacterial spores, which have the most resilient microbial survival strategy under extreme environmental conditions. As long as validated parameters are followed, successful elimination of viable cells, VBNC cells, and spores will be achieved (1, 2).

Conditions under non-sterile and aseptic manufacturing may be more permissive to VBNC bacteria. However, filtration, airflow, temperature, pressure, air particulates, intensive sanitization, and the use of preservatives are optimized during operation to reduce or eliminate microorganisms (2, 47). Furthermore, because manufacturing of pharmaceutical products comprises physical processes such as blending, compression, filtration, heating, encapsulation, shearing, tableting, granulation, coating, and drying, microbial cells are exposed to extensive environmental stresses. Microorganisms survive under those conditions, adapting to the lack of nutrients and other environmental fluctuations, by undertaking different survival strategies (45, 46, 48). Studies demonstrated microbial cells in pharmaceutical environments changing cell size and enzymatic and physiological profiles in response to environmental fluctuations (49–52). Similar responses were reported by bacteria exposed to drug solutions; significant morphological and size changes were observed (49). Bacterial cells spiked into different types of injectables products have shown different changes in their metabolism, enzymatic profiles, and structural changes that interfered with their identification using standard biochemical assays (49). Furthermore, bacteria undergoing starvation survival periods were capable of penetrating 0.2/0.22-micron-rated filters (50). Adaptation was also seen when bacteria developed resistance to the preservative systems and sanitizing agents incorrectly validated and used (4, 53, 54). The use of sub-optimal concentrations of disinfectants and preservatives resulted in situations where product and water quality were severely compromised. Increasing resistance to disinfectant sanitization was reported in pharmaceutical water systems with the following bacteria species isolated: *Pseudomonas aeruginosa*, *Pseudomonas fluorescens*, *Pseudomonas alcaligenes*, *Ralstonia picketti*, *Flavobacterium aureum*, *Acinetobacter lowffi,* and *Brevundimonas diminuta* (53). When compared to standard microorganisms, environmental isolates exhibited higher resistance to biocidal agents.

Why are culture-dependent methods unable to detect, and sometimes correctly identify, some microorganisms? Although these methods are valuable and do provide information on numbers, microbial genera, and species, they were developed for clinical samples such as human fluids or tissues, which are rich in nutrients and exhibit temperatures of 35–37 °C (20, 55). However, environmental samples, for example, raw materials, finished products, air, water, equipment swabs, and contact plates, taken from production facilities in compliance, are not rich in nutrients (oligotrophic), and their temperature fluctuates below and above the ambient temperature. The need for a stress recovery phase to recover microorganisms was demonstrated by longer incubation times and consistent higher recovery on low-nutrient media and temperatures lower than 35 °C. The recovery of microorganisms from pharmaceutical water samples and clean rooms was increased by using low-nutrient media (14, 15, 39, 40). Microbial numbers and diversity on agar media such as R2A and SCDA depended on incubation temperature, time, and nutrient composition. For instance, in purified water, bacterial isolates from R2A were identified as *Bradyrhizobium* spp., while isolates from SCDA were *Xanthomonas* spp. (14). Studies demonstrated the presence of a more abundant bacterial colony diversity on lower-nutrient media than standard media. In another study, Gram-negative bacterial colonies were observed on low-nutrient media but not on SCDA (40). No differences were found between the numbers of bacteria obtained on both media for air samples. However, surface samples exhibited a higher number of oligotrophic bacteria.

In conclusion, when proper contamination controls in pharmaceutical environments are implemented and validated, microorganisms undergo stressful conditions due to lack of nutrients and adverse environmen-

tal gradients. Therefore, in pharmaceutical environments microbial growth is sporadic and slow. Furthermore, microbial distribution is not homogenous. However, based upon FDA recall data and published scientific studies, when systems are out of control and environmental gradients are favorable, microorganisms contaminated finished products. Identification of microbial contaminants provided important information to track the sources of contamination, resulting in corrective actions that improved product quality and the system's optimization.

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