# What is an "Objectionable Organism"?

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## "Objectionable Organisms" - The Shifting Perspective

The definition of an "objectionable organism" has been under intense scrutiny recently. The critical nature of the issue is reflected by its presence in three separate citations in the current cGMP (italics added for emphasis):

- 21 CFR 211.84(d)(6) "Each lot of a component, drug product container, or closure with potential for microbiological contamination that is *objectionable in view of its intended use shall be subjected to microbiological tests before use.*"
- 21 CFR 211.113(a) "Appropriate written procedures, designed to prevent *objectionable microorganisms in drug products not required to be sterile*, shall be established and followed."
- 21 CFR 211.165(b) "There shall be appropriate laboratory testing, as necessary, of each batch of *drug product required to be free of objectionable microorganisms*."

So, 21 CFR 211.113 limits the discussion to "products not required to be sterile" and 21 CFR211.84 states that the concern is "microbiological contamination that is objectionable in view of its intended use" leaving 21 CFR 211.165 to require lab testing relevant to "drug product required to be free of objectionable microorganisms". This clearly defined concept of an "objectionable" organism then is one that is:

- Objectionable in view of the product's intended use, and;
- For products not required to be sterile

Perhaps the best way to examine the common understanding of the term "objectionable organism" is to look at some specific examples. To this end we will focus in on two organisms that have been generating a lot of interest recently, *Burkholderia cepacia* and *Bacillus cereus*. By using the recent activity in these two areas we can gain an appreciation for the "current" expectations, and hopefully compare this to "CGMP" – what is actually written in the 21 CFR 211.

## Burkholderia cepacia *as an "Objectionable Organism"*

A recent publication laid out the case for consideration of *Burkholderia cepacia* as an objectionable organism in non-sterile medications [1]. This publication is already being used as justification for 483 findings (personal communication) in a wide range of product types. While this organism is a clear pathogen when introduced into the air passages of a susceptible population, the case presented for categorization of *Burkholderia cepacia* as an "objectionable organism" in most non-sterile product presentations for general use remains unclear. This point is discussed in a "Letter to the editor" submitted to discuss the original article [2, 3]. The primary hazard of this organism seems to be to those with cystic fibrosis (or predisposed to pneumonia) and the use of inhaled medications. To this end, aqueous inhalants have been required to be sterile for over two decades [4]. Cases in the literature related to non-sterile products are extremely rare and would not seem to justify a requirement that this organism be absent in all non-sterile product presentations. Several of these cases are related to alcohol-free mouthwash (AFM) in cystic fibrosis patients (although there is also a clear concern to others who might be susceptible to pneumonia). Additionally there was reported an odd case of pneumonia from moisturizers in Spain [5]. On the basis of this case study, Irwin and Proce [6] recommended that non-sterile cosmetics not be allowed in critical care units – a prudent recommendation (protect the susceptible sub-population from exposure). Finally, a unique case of brain abscesses has been reported from which *Burkholderia cepacia* was isolated. In the complete absence of data (no relevant material was available to test) the authors suggest that the patient's ear drops might have been the source of the *Burkholderia cepacia*. [7].

From a historical perspective, there were no requirements for testing non-sterile products for microbial quality until USP introduced a test for the "Bacteriological Examination of Gelatin" (1942) [8]. However, most non-sterile medications in the US were not required to be checked for microbiological quality attributes until the introduction of the Microbial Limits Tests in 1970 [9]. In the late 1960's several outbreaks of disease were traced back to pathogen-contaminated medications, and this prompted increased attention to the microbial content of non-sterile drugs [10]. Later in the 1980's there was a series of articles appearing in the literature describing contamination by *Pseudomonas cepacia* (currently *Burkholderia cepacia*) [11, 12] and its survival in disinfectants [13-17]. This led to the addition of requirements in the 21 CFR to ensure that there are not objectionable organisms in product released to market (see above). This concern over all, potentially objectionable, contaminants is not addressed in the compendial Microbial Limits "Absence of Specified Organisms" tests. The USP is on record as early as 1982 verifying that the demonstration of "absence of objectionable microorganisms" is not the intent of the chapter. In a one page *Stimuli to the Revision Process* [18] the microbiology committee of the time states:

"The tests described in the *Microbial Limits Tests* <61> were not designed to be all-inclusive, i.e., to detect all potential pathogens. To accomplish this, an extensive text on laboratory detection of microorganisms would be required. The procedures in USP were designed to detect the presence of specific 'index' or 'indicator' organisms. Nevertheless, the present chapter does not preclude the detection of *Ps. Cepacia* – the organism requires subsequent differentiation. The chapter does not provide specific methods for this, nor does it provide procedures for detecting thousands of other potentially pathogenic organisms. Individual monographs include requirements for limits on total aerobic counts and/or absence of one or more of the four selected 'indicator' organisms. The chapter on *Microbial Limits Tests* provides methods to assure that one may test for those microbial requirements in the individual monographs..."

With the harmonization of the compendial tests, however, additional guidance was added to the informational chapter USP <1111> which states:

"…the significance of other microorganisms recovered should be evaluated in terms of the following:

- The use of the product: hazard varies according to the route of administration (eye, nose, respiratory tract).
- The nature of the product: does the product support growth? Does it have adequate antimicrobial preservation?
- The method of application.
- The intended recipient: risk may differ for neonates, infants, the debilitated.
- The use of immunosuppressive agents, corticosteroids.
- The presence of disease, wounds, organ damage.

Where warranted, a risk-based assessment of the relevant factors is conducted by personnel with specialized training in microbiology and in the interpretation of microbiological data. For raw materials, the assessment takes account of the processing to which the product is subjected, the current technology of testing, and the availability of materials of the desired quality." [19].

Let us be quite clear – in some presentations *Burkholderia cepacia* is a real danger, and this danger continues to the present day [20, 21]. This demonstrated danger is limited to a particular type of product and a specific patient population.

The harmonized compendial approach is describes a "risk analysis", rather than a "hazard avoidance" consideration. This remains perhaps the only regulatory treatment of "what is an objectionable organism?" available to the QC microbiologist.

It must be noted at this point that the experience with *Burkholderia cepacia* and its ability to grow in well-preserved formulation requires addition of a final consideration to "objectionable" – that is that the organism does not degrade the shelf-life stability of the product. So, to provide a bit more detail to our earlier definition of "objectionable organism"

- Objectionable in view of the product's intended use or to its shelf-life stability, and;
- For products not required to be sterile

The concern over a microorganism's potential impact on product shelf-life stability should not be overlooked. If an organism exists at

small concentration at manufacture in a non-sterile product, and this organism has the ability to proliferate in the medicine, its potential to degrade the shelf-life of the product is obvious. This is certainly a real concern with *Burkholderia cepacia* and a prudent activity might well be to challenge the preservative system of the non-sterile medication with this organism in addition to the compendial organisms normally used in the Antimicrobial Effectiveness Test. This challenge can be combined with the compendial test, and should be considered with the challenge of any other additional organisms of interest for this product type.

## Bacillus cereus *as an "Objectionable Organism"*

A second case study in the current discussion of "objectionable organisms" is the organism *Bacillus cereus*. *Bacillus cereus* is of concern as a food contaminant. The FDA Bad Bug Book states that an estimated 63,400 cases of self-limiting diarrheal disease occur annually (although only 3 – 6 per year were reported to CDC for the years 2005-2007 [22]. This disease is due to the production of enterotoxin, and the FDA states "The number of organisms most often associated with human illness is $10^5$ to $10^8$; however, the pathogenicity arises from preformed toxin." [22].

Recalls during the period of January 1, 2004 through July 1, 2012 among the pharma, medical device and cosmetics industries were analyzed for occurrences of recalls related to the presence of *Bacillus cereus* in the product. All were directly related to alcohol wipes. There were 50 recalls during this 90 month period – 46 of which occurred in 2011 and 4 in 2012. This analysis is a refinement and update of the recently published recall record review [23]. Forty-eight of these recalls are directly related to the problems at H&P Industries (under the brand name Triad). It should be noted that many of the recalls from H&P were associated with widespread GMP violations and the seizure of most of the company's products. This situation was sparked by a tragic fatality which occurred after an area of skin was prepared for injection using these wipes, and then the skin punctured for injection. The patient, a four-year-old child, developed septicemia from the same organism found in other samples from that lot (and many others) of sterile and non-sterile wipes (note – eight other fatalities were eventually linked to these products although the causal relationship was not able to be established [24]). Remember that this problem occurred in wipes that may have been labeled "sterile" but subjected to a substandard sterilization cycle or in wipes that were not sterilized nor labeled sterile. It might be reasonable to ask if the problem here is that *Bacillus cereus* is a pathogen or that the procedure required sterile preparatory wipes and any organism present in sufficient amounts would create a problem, (or the CGMP compliance of the manufacturer) [25]. These extreme situations are, fortunately, the exception rather than the rule, but the effects spread throughout the industry.

In addition to sanitizing an injection site with contaminated wipes, there are examples in the literature related to the risk of *Bacillus cereus* that bear attention. Wright et al. [26] documented a case of *Bacillus cereus* infection by the nasal route that mimicked the presentation of anthrax. They review some reports of sporatic pneumonia-like diseases described in the literature caused by *Bacillus cereus* and investigated a rapid, fatal case of anthrax-like pneumonia. This study determined that the infection was caused by a new strain of *Bacillus cereus* that was closely related to, but genetically distinct from, *B anthracis*. The strain contained a plasmid (an autonomously replicating DNA sequence) similar to that which encodes anthrax toxin and other known virulence factors. Immunological testing detected homologs of *Bacillus* anthracis virulence proteins were in infected tissues, likely contributing to the patient's death. This case study is of obvious concern.

Transmission of *Bacillus cereus* bloodstream infections by linens in the hospital setting has also been demonstrated [27, 28]. Sasahara et al looked at cases in a tertiary care facility in Japan. This team identified the strains of *Bacillus cereus* using Pulsed-field Gel Electrophoresis (PFGE) and found 4 distinct strains in the patients and in hospital linens and the laundry facilities. Following sanitary and hygiene improvements (sanitizing the equipment and instituting rigid handwashing requirements) the situation resolved [27]. Dohmae et al looked into an observed increase in *Bacillus cereus* during the summer months in Japanese hospitals [28]. These infections were identified first using multi-locus sequence typing (MLST) and then PFGE. Unique strains were seen in the hospital setting (different from food pathogens) and were traced through the towels and laundry in the hospitals. The towels were cleaned effectively using sodium hypochlorite at an external laundry.

While a hospital setting is, by its nature, extremely dangerous with a high likelihood of open wounds allowing direct entry to the bloodstream, these reports are worrisome examples of *Bacillus cereus* pathogenicity.

The increased recognition of *Bacillus cereus* pathogenicity and routes of infection have been the subject of recent reviews. Gaur [29] notes that pneumonia caused by *Bacillus cereus* is possible, but unlikely and restricted to immunocompromised patients. However, Bottone [30] presents a very different picture, discussing several cases of infection occurring through the oral and nasal routes among healthy individuals. While most of these were due to enterotoxin production, one case of bacteremia was strongly suspected to be due to the patient imbibing tea that was made from leaves contaminated with the organism. One possible explanation for this difference in perception between the two reviews might be an improved ability to correctly identify *Bacillus cereus* as present in the illness.

There were few reported cases of infections related to *Bacillus cereus* via the oral route separate from food poisoning cases in the literature reviewed (see [30] for descriptions of the rare events). However, there might be a consideration from looking at cases (discussed above) of patients acquiring pneumonia from *Burkholderia cepacia* from breathing in contaminated alcohol-free mouthwash (AFM) [31, 32]. This was presumably due to compromised patients inhaling aerosolized organisms while using the mouthwash. These cases are relevant because FDA has specifically highlighted the dangers of AFM contamination in podium presentations, most notably in regards to safety of cosmetics [33]. *Bacillus cereus* contamination of AFM might well be a cause for concern with AFM from the aspect of shelf-life stability (a concern that can be answered directly with empirical data on the ability of the organism to withstand the AFM preservative system, and its ability to grow in the formulation). However were no reports found in the literature of *Bacillus cereus* causing disease

through contamination of AFM. Having said that, this might be due to the difficulty in identifying *Bacillus cereus* as the organism has been implicated in disease through inhalation in at least one odd case (the authors of the study note "Very little is known about the molecular pathogenesis of these rare cases of fulminant infection caused by *Bacillus cereus* and related highly virulent strains.") [26].

While the potential danger of inhaling pathogenic strains of *Burkholderia cepacia* in AFM seem justified, and by extension, prudency may dictate concern over *Bacillus cereus* in this regard, the rare occurrence of this situation should not be extended to a blanket requirement that all non-sterile medications and personal care products must list *Bacillus cereus* as "objectionable", requiring a large amount of extra testing and potentially reducing the availability of (or at least increasing the cost of) all affected products with no demonstrable increase in the safety of the products.

To this concern must be added the realization of significant technical difficulties in the laboratory identification of the organism.

## Recovery and Identification of *Bacillus cereus*

Recovery of *Bacillus cereus* from samples has been a difficult task in the QC laboratory. Recently an efficient selective and recovery medium has been reported. BACARA (*Bacillus cereus* Rapid Agar – AES Chemunex) serves as the basis for the isolation of *Bacillus cereus* in the FDA Bacteriological Analytical Manual (BAM) [34]. Authors of the BAM chapter have recently published a study evaluating this medium which was shown to be useful in quantifying members of the *Bacillus cereus* group from food, in the presence of a large background of other bacteria [35]. A second agar is also available commercially (Brilliance *Bacillus cereus* Agar from Oxoid) as well as recently reported improvement in recovery media [36], but all seem subject to a significant false positive rate (recovery of organisms other than the target organism). This high false positive rate requires significant additional testing for identification, testing that in itself is subject to uncertainty (see below).

Identification of species within the genus *Bacillus* is problematic from both a genotypic and a phenotypic perspective. In particular, the species of *Bacillus cereus*, B. thuringenisis and *B anthracis* are so closely related that most identification systems cannot distinguish them [37]. While it would no doubt be useful to be able to distinguish a bioterrorism weapon from a biopesticide (*B. thurinenisis* has been sprayed on crops to minimize insect infestations), there seems to be no convenient way to do so. The FDA BAM recommends a series of 11 biochemical tests to distinguish among *Bacillus cereus*, *B. thuringiensis*, *B. mycoides*, *B. weihenstephanensis*, *B. anthracis* and *B. megaterium* after growth on selective and differential media. The BAM notes that these additional 11 tests "…are usually adequate for distinguishing the typical strains of *B. cereus* from other members of the *B. cereus* group. However, results with atypical strains of *B. cereus* are quite variable, and further testing may be necessary to identify the isolates."

A genotypic approach may be more precise and accurate. One method in particular, using multiple loci comparisons (Multi-Loci Sequence Typing or MLST) looks promising [38, 39]. While this technology is not currently cost-effective for the QC laboratory, there are contract laboratories proficient in the method servicing the relevant industries that can perform the analysis on suitable isolates.

## Prevalence in the Environment

While it is without question that rare cases of disease result from both *Burkholderia cepacia* and *Bacillus cereus*, this must be interpreted with an appreciation for the constant challenge we all receive on a day-to-day basis. These are common organisms in the environment and in our foods, we are accustomed to exposure.

One measure of this is illustrated in a study by A Ghosh [40] who looked at the occurrence of *Bacillus cereus* in the faeces of healthy individuals. The isolation frequency from hundreds of separate samples was approximately 14% - in other words, at least 14% of the healthy individuals in the study had consumed sufficient volumes of *Bacillus cereus* for the organism to pass through their system and remain in sufficient numbers to be isolated. A second study looked at a the safety profile of a substrain of *Bacillus cereus* (*Bacillus cereus* var. *toyoi*) used a probiotic microorganism in animal feed to promote growth and digestive health [41]. A final recent example is provided by milk, in which *Bacillus cereus* is a common contaminant, even after pasteurization [42].

Similarly, *Burkholderia cepacia* is a commonly encountered microorganism in the environment [43]. With improvements in genetic identification technologies the number of distinct members of the *Burkholderia cepacia* complex (Bcc) continues to grow, and recent in a recent study Mahenthiralingam [44] notes that there seems to be a clustering of subspecies specific to clinical isolation, environmental isolation and industrial processes. While some overlap among these groups exists, the picture on what type of Bcc organism causes disease and what type is completely benign is not clear (or even suitable to use as a biopesticide – [45]).

## Prevalence and Infectivity

Implicit in much of the discussion has been that the concern with patient health is due to the risk of disease. We have discussed the likelihood of infection from the perspective of the rarity of the reported cases for *Burkholderia cepacia* and *Bacillus cereus*. Another consideration is infectious dose. Just looking at the list of organisms that have been listed in the Bad Bug Book or been the subject of recall action (see Table 1) we do not get a reliable risk assessment of infection potential. An analysis based solely on whether or not the particular organism has been associated with a disease state does not give us this measure either. For example, the Fifth volume of Manual of Clinical Microbiology (1997) contains a chapter on the microorganisms of humans – in this chapter are several tables describing the microorganisms reported associated with different disease states [46]. Removing all viruses

**Table 1. "Objectionable" Organisms from FDA Bad Bug Book and Recall Analysis**

| | FDA Bad Bug Book | FDA Recalls |
|---|---|---|
| **Gram Negative Organisms** | | |
| Achromobacter xylosoxidans | | x |
| Aeromonas species | x | |
| Brucella species | x | |
| Burkholderia cepacia | | x |
| Campylobacter jejuni | x | |
| Cronobacter species (formerly Enterobacter sakazakii) | x | |
| Elizabethkingia meningoseptica | | x |
| Enterobacter cloacae | | x |
| Enterobacter cloacae | | x |
| Enterobacter gergoviae | | x |
| Enterobacter gergoviae | | x |
| Eschericia coli | | x |
| Francisella tularensis | x | |
| Klebsiella oxtoca | | x |
| Miscellaneous bacterial enterics | x | |
| Plesiomonas shigelloides | x | |
| Pseudomonas aeruginosa | | x |
| Pseudomonas aeruginosa | | x |
| Pseudomonas fluorescens | | x |
| Pseudomonas luteola | | x |
| Pseudomonas putida | | x |
| Pseudomonas spinosa | | x |
| Pseudomonas spp. | | x |
| Salmonella species | x | x |
| Serratia fonticola | | x |
| Serratia marcescens | | x |
| Shigella species | x | |
| Stenotrophomonas maltophila | | x |
| Vibrio cholerae Non-O1 Non-O139 | x | |
| Vibrio cholerae Serogroups O1 and O139 | x | |
| Vibrio parahaemolyticus | x | |
| Vibrio vulnificus | x | |
| Yersinia enterocolitica | x | |
| **Gram Positive Organisms** | | |
| Bacillus cereus and other Bacillus species | x | x |
| Clostridium botulism | x | |
| Clostridium perfringens | x | |
| Enterococcus spp. | x | |
| Listeria monocytogenes | x | |
| Staphylococcus aureus | x | x |
| Staphylococcus warneri | | |
| Staphylococcusintermedius | | x |
| Streptococcus spp | x | |
| **Yeast / Mold** | | |
| Acremonium mold | | x |
| Aspergillus spp | | x |
| Aspergillus sydowii | | x |
| Penicillium spp | | x |

from this consideration, we have approximately 190 microorganisms remaining in this 1997 analysis (which did not include either *Bacillus cereus* or *Burkholderia cepacia*). The authors split the microorganisms by site of culturing, frequency of occurrence and likely involvement in the disease state – these data are summarized in Table 2. The main point for this analysis is that there are many organisms associated with clinical samples – most of them occur only rarely and cannot be tied to a disease state. Association with a disease state cannot be the only criterion for determination that a microorganism is "objectionable". Even clear evidence of pathogenicity to a specific patient population by a specific route of administration should not be over-interpreted to the simplistic response of adding the organism to an "objectionable organism" list for all medications.

The established method of risk analysis (USP <1111>) is a reasonable starting point to determine the actual risk from an organism that may be found in a raw material or finished product. We also must take into account the numbers of organisms seen. The recall analysis is especially weak in this regard, as the magnitude of the contamination event is rarely listed in the enforcement report. For reasons discussed above, having a large number of contaminants in the product is a problem, indicating a failure of the manufacturing process controls or the preservative system and certainly raising concerns about shelf-life stability. However, numbers of bacteria also have an impact on the infectious dose of the potential pathogen. No patient should be exposed to large numbers of microorganisms directly introduced into the blood stream (as was most likely the case for the hospital reports). This is not relevant, however, to the question of if the organism involved is objectionable in consideration of the product's intended use (21 CFR 211.84(d)(6), 21 CFR 211.113(a) and 21 CFR 211.165(b) as discussed above). The GMP regulations clearly call for this type of risk analysis in determination of whether a particular organism is "objectionable".

## And What of the Human Microbiome?

There is an interesting "new" outlook on the relationship between human health and out microbial companions that bears some brief discussion. The Human Microbiome Project has undertaken a task to catalog our endogenous microorganism as "we rely on them to aid in nutrition, resist pathogens, and educate our immune system." [47]. It is becoming clear that "health" and "disease" are a bit more complicated than we previously thought. This work will no doubt

**Table 2. Overview of Microorganisms Found in Clinical Specimens**

| Sample Source | Total Count | Frequency of Isolation | | | Involvement in Disease | | |
|---|---|---|---|---|---|---|---|
| | | Common | Occasional | Rare | Common | Occasional | Rare |
| Ear | 24 | 0 | 10 | 14 | 2 | 19 | 3 |
| Eye | 32 | 1 | 14 | 17 | 3 | 15 | 14 |
| Genitourinary | 54 | 5 | 26 | 23 | 18 | 22 | 14 |
| Gastrointestinal | 82 | 6 | 41 | 35 | 27 | 27 | 28 |
| Respiratory | 71 | 10 | 37 | 24 | 20 | 33 | 18 |
| Skin, Wound, Burn | 75 | 5 | 43 | 27 | 11 | 39 | 25 |

confirm established understandings of frankly pathogenic organisms, but what will it do to our understanding of the complex relationship we as humans enjoy with our microorganisms, with whom we have co-evolved for millennia? [48].

## Conclusions

The question of "What is an Objectionable Organism?" might be one of the most pressing safety questions facing industrial microbiologists and regulators at the present time. This discussion should be approached from a solid grounding in the science with consideration for reasoned risk analysis.

This review has examined recent regulatory activity involving *Burkholderia cepacia* (a gram negative rod in the pseudomonad family) and *Bacillus cereus* (a gram positive, spore-forming rod). Careful consideration of available literature information suggests that there is evidence to support the consideration of both organisms as objectionable in some situations. This is not to say that they should be regarded as "objectionable" in all situations and one of the major points of the review is that we must have a consistent method for determination of "risk" in this regard.

What is an objectionable organism? A pure interpretation in regards to patient health would lead to the following requirements:

- Objectionable in view of the product's intended use or to its shelf-life stability, and;
- For products not required to be sterile

To this we also have to add a regulatory component. The organisms *Burkholderia cepacia* and *Bacillus cereus* are not objectionable in all non-sterile medications by the above definition. However, given recent events a finding of either organism in any non-sterile medication will most likely elicit interest from a regulatory agency. This is a proper response – it is up to the manufacturer to demonstrate that microorganisms present in non-sterile finished dosage forms are safe to release to market.

The discussion of "What is an Objectionable Organism?" is a critical one, and one that demands participation from everyone involved. FDA's proper orientation towards protection of the public above all else may lead to the conclusion that all organisms shown to cause any disease, or to be present in any disease state, will be considered objectionable in all medications. This position is logical if minimizing any potential risk to all possible populations is the only consideration. It is up to the manufacturer to provide evidence that the medications released to market are safe and to him falls the burden of showing that the microorganisms present in his non-sterile medication are not "objectionable". He must be prepared for this when challenged by FDA to defend the safety of his product.

## Acknowledgements

The author wishes to thank Don English, David Hussong, John Krowka, Karen McCullough and Ed Tidswell for helpful comment and review of this article during its preparation.

## Author Biography

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This article was printed in the September/October 2012 issue of *American Pharmaceutical Review*, Volume 15, Issue 6 pages 36-48.