

Implications of the Human Microbiome on Pharmaceutical Microbiology

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The human body is a composite of human cells that survive interdependently alongside complex, interconnected microbial populations, their genetic elements (genomes), and environmental interactions. Collectively, this is known as the human microbiome. Within this multifaceted ecosystem, microorganisms are believed to influence human physiology through processes related to digestion, immunity, development, and resistance to pathogens [1]. With the establishment of the Human Microbiome Project, our knowledge of the diverse span of microbial species within and across the human body has been significantly enhanced, revealing valuable insight into community niche specialization, genetic diversity, and the prevalence of indigenous opportunistic pathogens [2, 3].

The Human Microbiome Project (HMP) began in 2008 as a United States National Institutes of Health initiative. The core objective of this continuing project is to identify and characterize microorganisms associated with both healthy and diseased humans (the human microbiome) using a combination of culture techniques, metagenomics, and whole genome sequencing. As the Human Microbiome Project continues to improve our ability to characterize and understand the human microbiome, it has become evident that there are many implications to pharmaceutical microbiology that must be taken into consideration regarding the development, production, and use of therapeutic products, as well as the controlled environments within which medicinal products are produced.

For example, upon exposure to pharmaceutical therapies containing antimicrobial preservatives, the diversity and composition of the human microbiome can be compromised, potentially resulting in physiological changes or the overgrowth of opportunistic pathogens [4]. Conversely, the microbiome itself can also influence the human physiological response to pharmaceutical products, thus affecting the intended function of the product. A third consideration, which will be the focus for the remainder of this article, is that members of the human microbiome could be shed or deposited during the manufacturing process, thereby becoming an inadvertent source of contamination. This is a significant concern as members of the human microbiome are not always selected during the quality control analysis of pharmaceutical products. Moreover, the presence of certain microbial species from the human flora, such as indigenous opportunistic pathogens, has the potential

to considerably affect shelf-life stability and, more importantly, the safety of the product. Therefore, it is imperative that the significance of the human microflora be evaluated as potential sources of contamination.

In the pharmaceutical field, microbial species that pose a threat to product quality or patient safety are considered "objectionable microorganisms". There has, however, been significant scrutiny on what constitutes an objectionable microorganism [5]. In fact, the critical nature of this issue has been reflected by three separate citations described in the Code of Federal Regulations:

- 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."

Generally speaking, these citations indicate that objectionable microorganisms are those that are objectionable in view of the product's intended use, specifically concerning products that are not required to be sterile (creams, ointments, tablets, and so on). Other factors related to the risk of the microorganism include patient population (such as age and gender), patient health, dose, and application frequency of the medicine. With this definition in mind, to determine if members of the human microbiome can be considered objectionable in a particular product, one must consider the nature of the non-sterile product, the ability of the microorganism to degrade it, and the potential effects of the microorganism on the patient population using the recommended route of administration. A useful exercise to examine this is to monitor not only the numbers of microorganisms present on the operators in a facility, but also which species are frequently isolated as potential product contaminants.

This was recently examined through a review of the range, types, and patterns of microorganisms found in pharmaceutical cleanrooms. Generally, within cleanroom environments there are considered to be at least four main sources of facility contamination: people, surfaces (both inanimate and in relation to material and equipment transfer), air, and water [6-8]. Upon the analysis of an assortment of different grades of cleanroom, contamination was frequently associated with microbial flora from the human skin.

This was followed to a lesser extent by environmental and water contaminants [8]. Overall, the most commonly occurring microorganisms associated with pharmaceutical cleanrooms included species within the *Micrococcus*, *Staphylococcus*, and *Bacillus* genera [8]. It is noted that in this form of analysis, the microorganisms recovered will be affected by the metrological limitations of environmental monitoring methods, the types of culture media used, and the incubation parameters selected [9].

Following an initial analysis of possible sources of contamination, representative microbial species should then be examined to determine if they ought to be classified as objectionable microorganisms if found in the product. For this, a good microbial identification system should be used (one which is capable of species identification through either phenotypic or genotypic methodology). Several factors that should be considered include:

- **The nature of the product** – Can the product support microbial growth? Does it contain an effective concentration of antimicrobial preservatives? Is the product liquid-based or anhydrous?

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- **Whether the microorganism is likely to survive for long periods of time in the product**
- **The nature of the microorganism** – Is the organism an opportunistic pathogen? Will the addition of this microbial species at the administration site adversely affect the patient? Is the microorganism itself an indicator of other pathogenic species that might be present but not detected on this occasion? Keep in mind that some microorganisms should be regarded as indicator strains; that is they may indicate a concern with contamination from undesirable species. For example, the presence of Enterobacteriaceae may suggest a risk from *Escherichia coli* even if *E. coli* is not detected [10].
- **The absolute numbers of the microorganism recovered**
- **Microbial toxins** – Is the microorganism likely to release a toxin (exotoxin, enterotoxin or endotoxin) that could cause patient harm even if the microorganism is no longer viable?
- **The route of product administration** – What are the hazards associated with the route of administration? Is the target site normally sterile?
- **The intended recipient** – Will the product be used in immune-compromised patients? Is the patient currently suffering from any diseases or open wounds?
- **The use of other medications** – Is the patient currently using other medications that may result in diminished immunity?

Where warranted, these relevant factors should be analyzed in a risk-based assessment to uncover any potential threats that a particular organism may pose. It is useful to establish these risk assessments upfront and use them to cross-check against the types of microorganisms found. Any species considered to be a probable threat to the non-sterile product or patient should then be routinely screened for in addition to the testing already required by the Food & Drug Administration and the United States Pharmacopeia (USP).

Certainly the list of 'specified microorganisms' outlined in USP chapter 62 should not be taken as a definitive list. The chapter provides methodology for selected indicator organisms, but not all microorganisms which could be considered "objectionable". This article does not argue that the test panel should be increased, as the objectionable microorganisms found in each facility will be unique to that facility. Instead, the article argues that the use of a

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balanced risk assessment approach is the preferred methodology moving forward.

Where the above relates to a review of the common microorganisms recovered from environmental monitoring regimes or from direct product testing (intermediate and final product), consideration should also be given to the microorganisms recovered from pharmaceutical grade water systems (especially where the water is used to formulate the product). Furthermore, the same risk-based approach should be triggered when atypical and unusual microorganisms are detected which are not part of the established 'microflora' within the manufacturing facility. Thus, the risk-centric approach can be used as both a proactive and reactive tool.

Overall, pharmaceutical manufacturers are responsible for the quality and safety of all products that go to the market. Products should not only be safe with regards to chemical composition, but also free from objectionable microorganisms that may be a detriment to either the quality of the product or the welfare of the patient. It is up to each company's microbiological group to identify any potential source of contamination and associated risks, and to ensure that there are appropriate, valid testing methods that can ensure the absence of these objectionable microorganisms from the manufacturing process or within final product.

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