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Review Article

Bacillus: An Environmental Contaminant or Misunderstood Pathogen?

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Abstract

For many years, *Bacillus* species (spp.) recovered from patient samples were mostly considered as environmental contaminants and not agents of disease. Then came the current era when *Bacillus anthracis*, the bacterium responsible for anthrax, became listed as a potential bioterrorism agent. This ignited great interest in quickly identifying *B. anthracis*, which has translated into newer identification methods that also allow for classification of other *Bacillus* spp. There have been several reports of *B. cereus* in medical literature as the source of human disease, including its relationship to hospital-acquired infections. However, the actual role of *Bacillus* spp. other than *B. anthracis* and *B. cereus* as agents of human disease remains in question. A body of accumulating evidence from case reports to original research papers appears to indicate that several other *Bacillus* spp. have been implicated in severe and sometime life-threatening infections. In many cases these infections are seen to involve debilitated patients with a weaken immune response. This review collects a sampling of this literature to advance a greater understanding of the role of less frequently encountered *Bacillus* spp. in human infections. It by no means suggests that these findings are the only reports of esoteric *Bacillus* spp. infections. However, the relatively low number of reports may represent an unintentional underreporting of these species due to their ubiquitous nature in environments inside and outside of healthcare facilities. In words attributed to Sophocles - 'Look and you will find it - what is unsought will go undetected.'

Keywords: *Bacillus*; Hospital-acquired infections; Immunosuppression

Introduction

Much attention has been given to the genus *Bacillus* in recent years. This is primarily due to the genus member *Bacillus anthracis*, the causative agent of anthrax. Extensive study of *B. anthracis* characteristics has been completed primarily due to its potential use as a bioterrorism agent. This research has sparked some renewed interest in several other *Bacillus* species (spp.). This would include like *B. cereus* and *B. subtilis* both confirmed agents of human disease. These two bacilli, or rod-shaped bacteria, have become recognized as opportunistic human pathogens. Other *Bacillus* spp. recovered from patient samples are less well characterized in their ability to cause human disease. They are most likely considered an environmental contaminant. Traditional methods of *Bacillus* identification rely mostly upon extensive biochemical testing along with an examination of colony morphology on nutrient agar and Gram stain characteristics. Newer identification methods used in clinical microbiology laboratories such a real-time Polymerase Chain Reaction (PCR); [1] and (Matrix Assisted Laser Desorption/Ionization-Time of Flight (MALDI-TOF); [2] have afforded greater opportunities to investigate more esoteric *Bacillus* species associated with patient samples.

Immunosuppressed Patient Population

A question remains as to whether a *Bacillus* species recovered from a patient sample is a true pathogen or an environmental contaminant. Up until a few years ago, most *Bacillus* spp. were considered as environmental contaminants rarely being associated with disease when recovered from patient samples [3]. Today, *Bacillus* is classified as an opportunistic pathogen that is gaining increasing notoriety as a cause of severe infections in immunocompromised patients. *B. cereus* infections seen in this patient population include brain abscesses, coagulopathies, colitis, endocarditis, hemolysis, meningitis, respiratory tract infections, and septic shock [4]. Consequently, special consideration may be warranted to *Bacillus* species recovered in samples from debilitated patients with a depressed immune system response [5]. This would include cancer patients receiving either chemotherapy treatment alone or in conjunction with radiation therapy. It would similarly apply to patients receiving immunosuppressive drugs as a recipient of a transplanted organ (e.g. kidney) or from an acquired immunodeficiency. Since the 1980's, the immunosuppressed patient population has increased and may continue to increase, or at least remain steady, in the near future. These individuals are at increased risk of infections by microorganisms, bacterial, fungal, or viral that may be fatal [6,7]. Going forward, *Bacillus* spp. recovered from these patient samples deserve greater attention as agents of Healthcare-Acquired Infections (HAIs).

HAIs

HAIs are infections that are not present upon patient entry into a healthcare environment. They are a direct result of patient treatment occurring in a healthcare facility. Worldwide, HAIs are the most frequent adverse event in health-care delivery affecting hundreds

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of millions of patients. HAIs cause significant sickness (morbidity) and death (mortality) in both developing and developed countries. For every 100 hospitalized patients, 1 out of 7 patients (14.3%) in developed countries and 1 out of 10 (10.0%) patients in developing countries will be afflicted with at least one HAI at any one time [8]. HAIs are usually thought to be associated with treatment received in an inpatient setting. Diagnosis of HAIs are more difficult to establish for outpatients. The lack of hospital confinement makes it hard to distinguish between an infection caused by a contaminated medical device and an infection acquired by a patient while in a public venue (e.g., shopping center), which is referred to as community-acquired infection.

Bacillus Characteristics

The genus *Bacillus* represents a large group of aerobic gram-positive rods (Figure 1) belonging to the bacterial Phylum *Firmicutes*. Most *Bacillus* species are nonpathogenic bacteria feeding off dead or decaying organic matter [3]. They are a source of antibiotics including the topical ointment bacitracin, which is effective in preventing minor skin infections [9]. *Bacillus* spp. are ubiquitous in nature being found in a variety of land and water environments. They are very hardy microbes capable of withstanding a wide range of acidic-to-alkaline, low-to-high saline, and hot-to-cold conditions. *Bacillus* spp. can produce dormant endospores (spores) upon nutrient depletion or other unfavorable conditions. Their ability to form spores makes them resistant to effects of drying, heat, ultraviolet radiation, many disinfectants, and several other environmental stressors [9,10]. *Bacillus* spores are easily spread by a variety of means into man-made environments, including hospital operating rooms [3].

Spores in Disease Transmission

Actively growing bacteria/fungi are not required for disease transmission. Infection can be established by ingestion, inhalation, or implantation of spores into a susceptible host. *Bacillus* spp. spore are uniquely different from their actively growing (vegetative) counterparts. Spores possess several outer layers not likewise expressed by growing cells [11]. The chemical nature of *Bacillus* spp. spore coats makes it difficult to remove using aqueous based disinfectants [12]. Spore elimination can be very challenging. It usually requires a high level of prolonged disinfection. Additionally, *B. cereus* biofilms can generate highly resistant and adhesive spores that are not easily destroyed by either antimicrobials or routine cleaning procedures [13]. *Bacillus* spp. spores can last in the environment for long periods of time. For instance, spores of *Bacillus anthracis* can remain viable for decades [14].

The very nature of spores, being so environmentally widespread, makes it difficult to adequately control them once they are present in medical treatment areas. CDC's 2003 Guidelines for Environmental Infection Control in Health-Care Facilities [15] describes outbreaks and pseudo-outbreaks of *B. cereus* reported in several hospital units, including maternity, pediatric, intensive care, and bronchoscopy. These guidelines also identified several *B. cereus* contamination episodes that were secondary to (following) environmental contamination. Airborne transmission of spores generated from actively growing microbes was recognized as the source of these outbreaks. Here, spores are generated as local environmental conditions (e.g. nutrient depletion) become less supportive of their continued growth [9]. Likewise, *B. subtilis* spores are also very hardy. Strains of *B. subtilis* and closely related species are used to test sterilization techniques such as ethylene oxide gas sterilization [16] and ultraviolet germicidal irradiation [17].

Table 1: Uncommonly isolated *Bacillus* species associated with human disease (not a comprehensive list).

Species	Disease caused	Reports in Literature
B. circulans	Wound (abdomen - surgical) Cerebral spinal fluid (shunt) Endocarditis (prosthetic valve) Endophthalmitis Pericarditis Sepsis (fatal) Cellulitis (foot)	Logan NA, et al.(1985);[29] Roncoroni, A et al. (1985); [30] Krause A, et al. (1999); [31] Tandon A, et al. (2001); [32] Gurol Y, et al. (2008); [33] Alebouyeh M, et al. (2011); [34] Sanyal SK, et al. (2015); [35]
B. coagulans	Bacteremia	Banerjee C, et al. (1988); [28]
B. larvae	Cerebral spinal fluid (shunt)	Roncoroni A, et al. (1985); [30]
B. megaterium	Keratitis Skin (cutaneous) Brain abscess Pleuritis	Ramos-Esteban, JC et al. (2006); [36] Duncan, KO and Smith, TL (2011); [37] Guo FP, et al. (2015); [38] Crisafulli, E et. al. (2018) ; [39]
B. pumilus	Bacteremia Central venous catheter infection Skin (cutaneous) Neonatal sepsis Septic arthritis	Banerjee C, et al. (1988); [28] Bentur HN, et al. (2007); [40] Tena D, et al. (2007); [41] Kimouli M, et. al. (2012); [42] Shivamurthy VM, et al (2016); [43]
B. sphaericus	Lung (pseudotumor) Bacteremia (cancer) Bacteremia (pediatric cancer)	Isaacson P, et al. (1976); [44] Banerjee C, et al. (1988); [28] Castagnola E, et al. (2001); [45]

Bacillus cereus

Bacillus cereus is widely distributed in soil and water. It is pervasive in hospital settings. *B. cereus* has been isolated from several clinical samples, including wounds, blood, and sputum. It has become a larger problem for immunosuppressed patients. *B. cereus* is likewise a confirmed upper respiratory tract pathogen in the oral cavity in immunosuppressed patients [5]. The prevalence of these infections is now thought to be greater than formerly recognized. Oral cavity colonization may occur through ingestion of vegetative *B. cereus* cells in contaminated food or inhalation of spores. Severe lower pulmonary tract infection has been similarly documented. Some *B. cereus* strains have even been shown to have *B. anthracis* toxin genes [18].

B. cereus has also been implicated as the cause of HAI-related bloodstream infections. An outbreak of *Bacillus* spp. bacteremia in a Hong Kong university-affiliated hospital was tracked back to contaminated bed linen [19]. Testing of 113,207 blood cultures from 43,271 patients revealed 978 (0.86%) positive samples from 744 (1.72%) patients containing *Bacillus* species. The *Bacillus* cereus group accounted for 14 of 87 (16.1%) patient isolates and were phylogenetically related to 9 linen sample isolates. The linen laundry protocol was subsequently changed to prevent further episodes of *Bacillus* spp. transmission. This study underlines the importance of controlling *B. cereus* spores, which were the most likely present on contaminated linen and capable of surviving the previous sheet-laundering procedure.

B. cereus also causes a benign and self-limiting foodborne gastroenteritis in healthy individuals. As such, it has become the

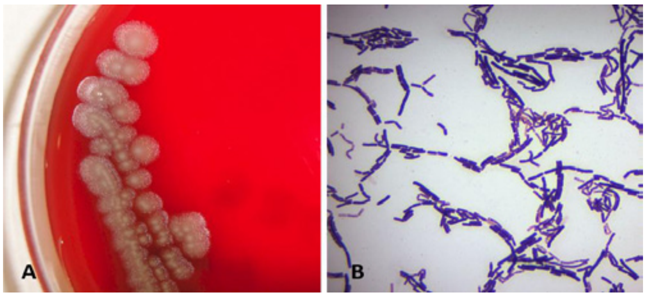

Figure 1: *B. megaterium* colonies grown on 5% sheep blood agar (A) and Gram stain (B) demonstrating Gram-positive rods viewed under 100X total magnification (Images provided by author).

second most common cause of bacterial foodborne outbreaks in France and third largest source in European medical facilities [20]. This same group established that several hospitals in France were cross contaminated by an identical strain of *B. cereus*. The same *B. cereus* strain was recovered from several different patients and environmental sources in 9 separate hospitals over a 2-year monitoring period. The occurrence of both intra and inter-hospital contamination by the same bacterial strain was considered the first documented evidence of a nosocomial epidemic caused by *B. cereus*. Prior to this study, infection of different patients with the same *B. cereus* strain had not been documented at these same facilities. To complicate matters further, *B. cereus* is resistant to several first- and second-line antibiotics. Most *B. cereus* isolates are resistant to penicillins and cephalosporins via β-lactamase production. Resistance to erythromycin, tetracycline, and carbapenem has also been noted [5].

Bacillus subtilis

Literature reports of human infection by *B. subtilis* are much less frequent than *B. cereus*. Only two reports of human disease attributed to *B. subtilis* could be found after reviewing 150 manuscripts using PubMed search engine (US National Library of Medicine/ National Institutes of Health). Key words included "*bacillus*" and "subtilis" used in conjunction with either "infection" or "disease". In 1996, Wallet et al., reported *B. subtilis* as a cause of cholangitis in polycystic kidney and liver disease [21]. In 2018, Tsonis et al documented a case of spontaneous cerebral abscess due to *B. subtilis* in an immunocompetent male patient. This group indicated that *B. subtilis* recovered from central nervous system specimens should not be merely thought of as laboratory contaminant [22]. More often, reports were found indicating potential use of *B. subtilis* to prevent human disease. *B. subtilis* possesses probiotic properties, immunomodulation effects, antifungal capabilities, inhibits ulcerative colitis and associated cancers, and a parasitic vaccine [23-27]. For example, *B. subtilis* spores have also been used to stimulate mice alveolar macrophages to protect against infection with respiratory syncytial virus A2 [24].

Other *Bacillus* Species

Reports of *Bacillus* spp. other than *B. cereus* and *B. subtilis* are more infrequent (Table 1). In 1998, Banerjee, et al retrospectively examined the records of 18 febrile patients diagnosed with *Bacillus* bacteremias over a nine-year period [28-30]. The isolates included *B. cereus* (44.4%), *B. circulans* (16.7%), *B. subtilis* (11.1%), *B. pumilus* (11.1%), *B. licheniformis* (11.1%), *B. sphaericus* (5.5%), *B. coagulans* (5.5%), and six undetermined species (33.3%). All 18 patients had underlying health conditions including lymphoma or leukemia (83.3%) and breast cancer (16.7%). Nine patients had decreased white blood cell counts (neutropenia), seven patients had indwelling Hickman catheters, and 14 patients had recently undergone chemotherapy treatment [31-34]. Contaminated Hickman catheters removed from four patients were examined by both Scanning Electron Microscopy (SEM) and Transmission Electron Microscopy (TEM). One catheter demonstrated *Bacillus* microbes associated in a biofilm along with gram-positive cocci and glycocalyx coating. The author's noted that *Bacillus* spp. should be recognized as a bacterial pathogen in compromised hosts.

In 2006, Ozkocaman et al reported results form a 5-year study of 350 episodes of bacteremia among patients being treated for hematologic malignancies [35-40]. Coagulase-negative staphylococci (CoNS) and *Staphylococcus aureus* accounted for 65.1% of the bacteremic episodes while gram-negative pathogens like *Escherichia coli*, *Acinetobacter baumannii* and *Pseudomonas aeruginosa* were responsible for 34.8% of these infections. *Bacillus* spp. were determined to be the cause of 3.4% of all bacteremias. Two deaths were noted. The identified species included *B. cereus*, *B. pumilus*, and *B. licheniformis*. The author's remarked that *B. cereus* and *B. licheniformis* may be new gram-positive pathogens causing serious infection in neutropenic patients.

Most recently, Chun et al examined bacterial types recovered from pediatric blood cultures to determine if they were simply contaminants or clinically significant isolates causing disease [41-47]. A retrospective review of microbiology laboratory reports and patient medical records was conducted for over 76,000 recovered blood culture isolates over a period of 10 years. Seven hundred and fifty isolates were determined to be true pathogens and not blood culture contaminants. Rec *B. pumilus* overed blood culture isolates included *Aerococcus* spp., *Bacillus* spp., *CoNS* spp., *Corynebacterium* spp., *Micrococcus* spp., viridans group *Streptococcus*, and *Proprionibacterium* spp. Results indicated that viridans group *Streptococcus* spp. had the greatest clinical significance as a true pathogen in 46.2% of all cases followed by *Bacillus* spp. at 27.7%, CoNS at 23.8%, and for *Corynebacterium* spp. At 19.0%. Of the recovered *Aerococcus* spp. (n = 14) and *Micrococcus* spp. (n = 8) neither bacterium was considered as a true pathogen. It should be noted that the CoNS pathogen rate for patients with malignancies was substantially higher at 43.7% when this patient populated was shredded out separately from all cases. The number of patients with malignancies demonstrating *Bacillus* spp. was not similarly reported. This study made a note of cases of *Bacillus* spp. related bacteremia have been reported in invasive infections, including *B. cereus* bacteremia and meningitis occurring in immunocompromised children. This group concluded that underlying patient conditions such as malignancy, preterm birth, immune system status, congenital heart disease should be considered when determining the true pathogenicity of an isolate whose importance is ignored and discarded as merely a contaminant.

Conclusions

This review is by no means comprehensive in its scope. It does however bring to light the importance of determining the true nature of a *Bacillus* spp. when recovered from a patient sample. Collectively, the evidence presented within suggests that several *Bacillus* spp, other than *B. anthracis*, represent a danger to patients, especially those with underlying health conditions (e.g cancer). More conclusive study is needed to establish a greater relationship of these lesser known *Bacillus* spp. to human disease. It is hoped that further improvements in contemporary detection methods along with increased reporting of *Bacillus* spp. isolates found in patient samples will lead to a better understanding of this likely pathogen group. Until then, caution is warranted before simply discarding a *Bacillus* spp. isolate as merely a patient sample contaminant.

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