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To Alessia, my queen, and Giorgia, my princess

Chapter

BACILLUS CEREUS PNEUMONIA

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ABSTRACT

Bacillus cereus is a Gram positive/Gram variable environmental rod, that is emerging as a respiratory pathogen; particularly, pneumonia it causes may be serious and can resemble the anthrax disease.

In fact, the organism is strictly related to the famous *Bacillus anthracis*, with which it shares genotypical, fenotypical, and pathogenic features.

Treatment of *B. cereus* lower airway infections is becoming increasingly hard, due to the spread of multidrug resistance traits among members of the species.

Hence, the present chapter's scope is to shed a light on this bacterium's lung pathogenicity, by depicting salient microbiological, epidemiological and clinical features it shows. Also, we would like to explore virulence determinants and resistance mechanisms that make *B. cereus* a life-threatening, potentially difficult-to-treat agent of airway pathologies.

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INTRODUCTION

The genus *Bacillus* includes spore-forming species, strains of which are not usually considered to be clinically relevant when isolated from human specimens; in fact, these bacteria are known to be ubiquitously distributed in the environment and may easily contaminate culture material along with improperly handled sample collection devices (Miller, 2012; Brooks, 2001).

Bacillus anthracis is the prominent agent of human pathologies within the genus *Bacillus*, although it is uncommon in most clinical laboratories (Miller, 2012). It is a frank pathogen, as it causes skin and enteric infections; above all, however, it is responsible for a serious lung disease (the 'anthrax') that is frequently associated with bloodstream infection and a high mortality rate (Frankard, 2004).

Instead, *Bacillus cereus* is an environmental organism that is known to be widely and ubiquitously distributed in nature; it mainly inhabits soil and vegetation, as ecological niches (Hoffmaster, 2006; Forsberg, 2011; Strauss, 2001; Savini, 2009); particularly, it has been found to contaminate rice, with *B. cereus*-related intoxications being frequently associated with the ingestion of this food (Brooks, 2001).

As a consequence of the organism's ubiquity, the clinical role it potentially plays is often disregarded when *B. cereus* is cultivated from clinical samples; usually, in fact, it is believed to behave as a mere, harmless contaminant, so strains are commonly labeled as non-pathogen isolates and simply dismissed (Frankard, 2004). Nonetheless, this bacterium is not only responsible for a transient colonization in man; indeed, it may cause diseases, even serious and lethal (Brooks, 2001).

MICROBIOLOGY

B. cereus is an aerobic or facultatively anaerobic, motile or nonmotile, spore-forming, rod-shaped organism (Hoffmaster, 2006; Strauss, 2001; Miller, 2012); as mentioned in the *Introduction*, it is ubiquitously distributed in the environment, so it can be easily found in dirt, air, soil, water, stools, and plant surfaces; the latter typically include rice (Katsuya, 2009; Miller, 2012).

In the routine laboratory practice, *B. cereus* appears as positive or variable at Gram staining (although it is usually labeled as a Gram positive rod), and forms hemolytic (Fig. 1-2) or nonhemolytic colonies (Hoffmaster, 2006)

The organism is relatively resistant to heat due to the formation of spores; therefore, it grows easily during food storage and may be responsible for food poisoning and related emetic or diarrheal syndromes (Katsuya, 2009). Exactly, it produces toxins (see *Pathogenic determinants*) that can be found in the food, or be produced in the gut after the ingestion of *B. cereus* contaminated products; in both cases the result is a foodborne enteric intoxication (Brooks, 2001).

TAXONOMY

According to the current taxonomic classification of the genus *Bacillus*, *B. cereus* should be indicated as *B. cereus sensu strictu*; in fact, the latter is part of a group of environmental microorganisms, which is classified as *B. cereus* group (or *B. cereus sensu lato*) (Savini, 2012; Forsberg, 2011).

B. cereus (sensu lato) comprises the six species *B. anthracis* (the most famous within the group, being known worldwide as the aetiologic agent of the anthrax), *B. cereus sensu strictu* (mostly associated with emetic and diarrheal food poisoning), *Bacillus mycoides*, *Bacillus pseudomycoides*, *Bacillus thuringiensis* and *Bacillus weihenstephanensis* (Savini, 2012).

These species share phenotypical features, so they cannot be distinguished on a metabolic, biochemical or visual basis; instead, discrimination is successfully achieved by using molecular, genome-based methodologies. Nontheless, they are genotypically (16S rRNA) very closely related each other, and there is evidence, particularly, that *B. anthracis*, *B. cereus* (*sensu strictu*), and *B. thuringiensis* may indeed represent one only organism (Bottone, 2010; Savini, 2012). Also, the spore surface antigens of *B. cereus* share epitopes with *B. anthracis* spores, as it has been shown through cross–agglutination assays (Bottone, 2010).

For convenience, we are referring to the organism asto *B. cereus*, from now on, instead of *B. cereus sensu strictu*.

Unfortunately, however, more than one published work dealing with this species did not provide any molecular-based identification; conversely, a number of isolates have been characterized through a phenotypical investigation, only, in the past decades (Uchino, 2012); in these cases, then, we assume that authors refer to *B. cereus sensu strictu*, but it is clear that the

literature is in part fragmentary and confused due to the unclear or unreliable methods employed to characterize *B. cereus* group isolates.

EPIDEMIOLOGY

B. cereus is an ubiquitous environmental rod; exactly, its natural reservoir is represented by decaying organic matter and vegetables, fresh and marine waters, along with the intestinal tract of invertebrates from which soil and food may become contaminated (leading to human gut transient colonization) (Bottone, 2010). When bacilli come into contact with organic matter or within an insect or animal host, they may bacilli lose their flagella, attach to the arthropod enteric epithelium, and sporulate (Bottone, 2010).

B. cereus also has a saprophytic life cycle where spores germinate in the soil, with the production of vegetative bacilli, that could then sporulate, keeping the cycle; following host defecation or death, cells and spores are released into the soil, where vegetative organisms may sporulate again, then surviving until the uptake by another host (Bottone, 2010).

Survival of *B. cereus* in the environment is then strictly related to the spore; the latter is in fact resistant to extreme conditions including heat, radiation, drying, freezing, and may be considered to be the infective agent for this organism (Bottone, 2010).

In the food industry, such spores are particularly troublesome since they can be refractory to pasteurization and gamma radiation; moreover, their hydrophobic nature permits them to adhere to surfaces, and this feature enables the bacterium to spread to all kinds of food (Bottone, 2010). Given the ubiquitous distribution of *B. cereus* in food products, therefore, the organism is ingested in small amounts and becomes part of the transitory human enteric flora; it is unclear, however, if the recovery of *B. cereus* from man stools is a function of germinating spores or the growth of vegetative cells (Bottone, 2010).

DISEASE

B. cereus has been widely observed to cause human infections, mostly foodborne intoxications (Hoffmaster, 2006); particularly, such syndromes are represented by self-limited ailments and include an emetic presentation (due to an emetic toxin and frequently associated with the ingestion of fried rice) and a diarrheal one (due to enterotoxins and commonly related to contaminated meat or sauces) (Brooks, 2001; Forsberg, 2011; Frankard, 2004). Typically, when a large amount of rice is cooked, and then gets cold slowly, spores may find the conditions permitting them to germinate; as a consequence, the vegetative cells produce the toxin, that can be present in the food before the ingestion, or be formed in the intestine, after being introduced with food (Brooks, 2001).

Aside from food poisoning, several infectious processes have been attributed to *B. cereus* in the past and recent years, including periodontal diseases, ocular infections (endophtalmitis, panophtalmitis and keratitis, that develope after the microorganism introduction into the eye due to the occurrence of a traumatic event), skin as well as post-operative and post-traumatic wound infections (with or without bone involvement), osteomyelitis, necrotizing fasciitis, salpingitis, meningitis, endocarditis, bacteremia (Katsuya, 2009; Hoffmaster, 2006; Frankard, 2004; Brooks, 2001; Savini, 2009). Again, a *B. cereus* strain was cultivated from the necrotic ulcer on the arm of a rhabdomyosarchoma patient (Savini, 2009).

Immunocompromised subjects (hematologic hosts, premature newborns, critically ill and debilitated patients, along with those recovering from surgery) are mainly affected; among these categories, particularly, septicemia, related multiorgan failure and a fatal outcome are usually observed in neutropenic hosts (Katsuya, 2009; Hoffmaster, 2006; Frankard, 2004; Jevon, 1993; Funada, 1991).

Nonetheless, immunocompetent people (showing niether apparent predisposing conditions nor risk factors) can develop serious infections caused by this organism, too (Table 1) (Carbone, 1985).

In the context of non-enteric pathologies, *B. cereus* has been known to act as an unusual agent of pneumonia; however, although rare, such a condition

may be severe, as it can mimic anthrax-related pictures, it mostly involve immunocompromised subjects and potentially leads patient to exitus (Feldman, 1974; Bekemeyer, 1985; Coonrod, 1971; Stopler, 1965) (Table 1).

Apparently healthy people with neither immunocompromission nor underlying predisposing conditions can develop *B. cereus* pneumonia, as well, although that of welders seems to be a category of subjects that show an increased (professional) risk to acquire *B. cereus* airway infections (owing to poorly understood reasons) (Miller, 2012; Hoffmaster, 2006).

Table 1. Recent *B. cereus* pneumonias from the published literature (Hoffmaster, 2006; Sue, 2006; Miller, 2012; Katsuya, 2009; Wright, 1997; Frankard, 2004; Carbone, 1985)

Case number	Risk factor/comorbidity	Outcome
1 case	Welder (immunocompetent)	Non-fatal
2 cases	Welder (immunocompetent)	Fatal
1 case	Muller operator (immunocompetent)	Fatal
1 case	Leukemia	Non-fatal
2 cases	Leukemia	Fatal
1 case	Aplastic anemia	Fatal
1 case	None	Fatal

AIRWAY INFECTION

B. cereus has been known to rarely cause lung diseases; nevertheless, reports of pulmonary infections mimicking anthrax have been attributed to strains harboring *B. anthracis* toxin genes (see *Pathogenic determinants*) (Bottone, 2010).

Airway ailments caused by this organism mostly affect compromised hosts; particularly, several clinical and radiological presentations have been associated with *B. cereus* low airway infections, including unilateral/bilateral pneumonia, or multicentric alveolar infiltrates (even confluent); such conditions have been mostly associated with dyspnea, hemoptysis, and productive cough as major signs and symptoms (Carbone, 1985; Avashia, 2007; Miller, 2012; Wright, 1997).

Pleura may be involved, as well, with pleura-related pathologic processes being variably represented by effusions or empyema (Carbone, 1985; Avashia, 2007; Miller, 2012; Wright, 1997).

Furthermore, pulmonary edema and perihilar infiltrate have been reported in the published literature, as well as pneumonia-associated clinical pictures (other than respiratory) including enteric and cutaneous disorders (nausea, vomiting, hematemesis, diarrhea and bullous skin lesions).

Finally, general signs may be observed, such as fever, chills, and leukocytosis, along with a potential septicemic spread of microorganisms; globally, the outcome of *B. cereus* pneumonia is often unfavourable, as the disease is severe and potentially leads patients to death, unfortunately (Table 1) (Carbone, 1985; Avashia, 2007; Miller, 2012; Wright, 1997).

As most respiratory infections by *B. cereus* are fatal, a large amount of autoptic data is available and has permitted to better understand the underlying histopathologic processes which *B. cereus* pneumonia relies on. In this context, autopsies have alternatively revealed necrotizing pneumonia and bronchopneumonia (where numerous rods were observed inside both alveoli and pleura) (Carbone, 1985; Avashia, 2007); again, abundant serosanguinous fluid has been found in the pleural cavities (Wright, 1997), as well as fibrinopurulent material which was adherent to the pleural surface (Carbone, 1985); other findings are represented by hemorrhage and edema of the pleura and the interlobular septa, intra-alveolar edema and intra-alveolar flogistic infiltrate (Wright, 1997).

Concerning extra-pulmonary post-mortem findings, splenic congestion has been documented (congestion of the spleen was found to be associated with the presence of immunoblasts), along with the recovering of Gram positive bacilli in the context of the serous fluid overflowing from the bullous skin lesions (Wright, 1997).

A fatal tracheobronchitis (with related pneumonia) was described in 2001, and involved an immunocompromised, hematologic patient with an underlying aplastic anemia; the clinical presentation was initially represented by chest pain and yellowish sputum, but the infection then showed a rapid progression to an anthrax-like pneumonia (Strauss, 2001). Post-mortem, histopathology showed a severe pseudomembranous inflammation of both trachea and the bronchial tree; also, diffuse alveolar damage and hemorrhage were documented, while fiberoptic bronchoscopy demonstrated the presence of a seriously inflammed tracheal and bronchial mucosa, along with the obstruction of the entire visible bronchial system (bilaterally) caused by diphtheria-like membranes (complete bronchoscopic removal of which was prevented by mucosal bleeding) (Strauss, 2001).

In general, *B. cereus* tends to cause necrotizing infections, owing to the expression (in the ambit of the emetic or diarrheal syndromes) of the

enterotoxin, as well as of the phospholipases (see *Pathogenic determinants*); although the role of these molecules in the pathogenesis of *B. cereus* extraintestinal diseases is unclear, however, it is likely that they contributed to the formation of the pseudomembranes described (Strauss, 2001).

Although *B. cereus* pneumonia has been mostly reported to be a sporadic event, nosocomial outbreaks have been described; particularly, such cases were due to contamination of hospital linen or inadequate sterilization of respiratory circuits (Katsuya, 2009). However, given the ubiquitous distribution of *B. cereus* in the environment, it is usually hard to clearly understand the true source of a pulmonary infection and, although a concurrent diarrhea may be suggestive of a foodborne rather than environmental origin, the disease natural history usually remains unclear (Katsuya, 2009).

PATHOGENIC DETERMINANTS

The pathogenicity of *B. cereus* is known to be intimately associated with production of tissue-destructive/reactive exoenzymes; among these secreted toxins are three distinct phospholipases, an emesis-inducing toxin (which is responsible for the vomiting syndrome), and three pore-forming enterotoxins: the cytotoxin K, the hemolysin BL (HBL), and a nonhemolytic enterotoxin (NHE) (Bottone, 2010; Strauss, 2001).

In the small intestine, vegetative cells that have been ingested as viable forms or spores induce a diarrheal syndrome (that is thought to be related to HBL and NHE), while the emetic toxin (a plasmid-encoded cyclic peptide called ‘cereulide’) is ingested preformed or is produced in foods (e.g., milk, rice, and pasta) where it is a metabolic product of *B. cereus* growth (Bottone, 2010).

Also, it is likely that such enzymes contributed to the formation of the diphtheria-like pseudomembranes observed in the context of the tracheobronchitis case (Strauss, 2001).

B. cereus is genotipically very close to *B. thuringiensis*, and many strains of these two species are phylogenetically strictly related to *B. anthracis*, as well (Wright, 1997); particularly, *B. cereus* isolates sharing genome similarity with *B. anthracis* tend to be of clinical rather than environmental origin and may seldom carry *B. anthracis* virulence genes (Hoffmaster, 2006).

These have been observed to encode extracellular molecules (i.e. the *B. anthracis* lethal factor, the edema factor, the protective antigen and the capsule, that are the main pathogenic factors expressed by this pathogen) and

other pathogenic traits involved in a clinical phenotype with many features of the inhalation anthrax (Wright, 1997).

Particularly, *B. cereus* strains producing the anthrax toxic genes *pagA*, *lef*, and *cya* (encoding for the protective antigen, the lethal factor and the edema factor, respectively) have been responsible for fatal cases of lung infection (Forsberg, 2011; Avashia, 2007; Wright, 1997).

Alternatively, *B. anthracis* toxin genes harbored in severe metalworker pneumonia–related *B. cereus* isolates seem to play an unclear role in the pathogenicity of *B. cereus* lung diseases (Hoffmaster, 2006); in fact, plasmids carrying *B. anthracis* genes appear not to be required for serious lung infections, as other isolates from severe cases have been found not to harbor plasmid pXO1 (carrying *pagA*, *lef*, and *cya* genes) (Bottone, 2010).

Toxin producing *B. anthracis* elaborates a capsule (production of which is encoded by the genes *capA*, *capB* and *capC*) which is essential for the pathogenesis of the anthrax disease; similarly, toxin expressing *B. cereus* strains produce a capsule, as well, that takes part in the pathogenesis of the inhalation anthrax-like picture (Wright, 1997; Sue, 2006). In this context, *B. cereus* strains G9241, 03BB87 and 03BB102 (isolated from cases of severe inhalation anthrax-like pneumonia) share relevant homology with *B. anthracis* genome sequences and harbors almost the whole pXO1 anthrax virulence plasmid. However, while *B. anthracis* capsule (encoded by the pXO2 virulence plasmid) is composed of poly-D-γ-glutamic acid, the capsule expressed by strains G9241, 03BB87 and 03BB102 shows a polysaccharide structure (instead of the poly-D-γ-glutamic acid composition) (Hoffmaster, 2006; Forsberg, 2011; Wright, 1997).

Strains G9241 and 03BB87 additionally carry the circular plasmid pBC218, that is thought to be involved in the formation of the polysaccharide capsule (Forsberg, 2011); instead, strain 03BB102 has been detected to harbor the *capA*, *capB* and *capC* genes (that are needed for the *B. anthracis* capsule synthesis), although this strain's capsule does not show the poly-D-γ-glutamic acid composition (it is a polysaccharide capsule, similar to that of strains G9241 and 03BB87) (Hoffmaster, 2006).

Hence, while *B. anthracis* virulence has been widely studied in the past decades, it is clear that the pathogenic elements involved in *B. cereus* infections are poorly understood, and have to be further investigated and more clearly defined.

In inhalational anthrax, it has been clarified that pathogenesis involves phagocytosis of bacterial spores by pulmonary dendritic cells, spore transport within them to the tracheobronchial lymph nodes (where the spores

germinate), replication of vegetative bacteria that secrete the edema toxin and the lethal toxin (causing extensive tissue injury, hemorrhage, and extension of the process through the mediastinum), dissemination through the bloodstream and hemorrhagic meningitis (that is the major cause of death) (Walker, 2011). So, it is presumable that toxin producing *B. cereus* strains may share the same (or similar) pathogenic mechanisms as a pathologic basis for the development of organ impairment and tissue necrosis (Katsuya, 2009).

ANTIBIOTICS AND THERAPY

Therapeutic options against *B. cereus* diseases usually revolve around the antibiotic susceptibility profile of the isolated (and correctly identified) strain, although species-specific criteria for testing and interpreting the *in vitro* response to drug have not been defined, yet. In the setting of a suspected *B. cereus* infection, however, empirical antimicrobial treatment may be indispensable pending the antibiotic sensitivity testing results (Bottone, 2010). In general, most *B. cereus* isolates are resistant to penicillins and cephalosporins as a consequence of β-lactamase production; in particular, it is likely that resistance to penicillin, ampicillin and cephalosporins should be considered to be constant (Fig. 3), nowadays, like that to trimethoprim (Brown, 2012).

A chromosomal metallo-β-lactamase (MBL) is widespread in *B. cereus* (the enzyme is called 'BcII') (Brown, 2012). Of interest, *B. cereus* MBL has been the first enzyme of this category that was discovered, in 1966 (Sabath, 1966), while MBLs were later described in *Stenotrophomonas maltophilia*, *Aeromonas* spp., *Bacteroides fragilis*, *Acinetobacter* spp., *Pseudomonas aeruginosa*, and some flavobacteria, and have been known to inhibit all β-lactams except for the monobactam (aztreonam, to which Gram positive organisms are however intrinsically resistant) (Savini, 2009). Furthermore, *B. cereus* usually produces Bush group 2a penicillinases I and III, that typically hydrolyze penicillins and are inhibited by clavulanic acid (Savini, 2009).

Among betalactams, then, *B. cereus* strains have been found to potentially express resistance to all among ampicillin, penicillin G (and penicillins in general), ampicillin-sulbactam, amoxicillin-clavulanic acid, oxacillin, as well as to cephalosporins (including third and fourth generation compounds such as ceftriaxone, cefotaxime, and cefepime) (Fig. 3) (Wright, 1997; Katsuya, 2009; Frankard, 2004; Miller, 2012; Bottone, 2010; Savini, 2009).

Resistances may also include those to cotrimoxazole, clindamycin, erythromycin, tetracyclines, and carbapenems (imipenem, meropenem), so it may be difficult to choose a proper empirical treatment; nonetheless, *B. cereus* has been found to be alternatively susceptible to these compounds (Fig. 4), as well as to tigecycline (Wright, 1997; Katsuya, 2009; Frankard, 2004; Miller, 2012; Bottone, 2010; Brown, 2012; Savini, 2009).

Molecules to which isolates may be susceptible also include fluoroquinolones (ciprofloxacin, levofloxacin, moxifloxacin), chloramfenicol, aminoglycosides (amikacin, gentamicin) and glycopeptides (vancomycin) (Wright, 1997; Katsuya, 2009; Frankard, 2004; Miller, 2012; Leff, 1977), and a recent *in vitro* study showed 100% sensitivities of 42 strains to rifampin, daptomycin, and linezolid (Bottone, 2010); in agreement, strains from our laboratory collection showed low MICs for daptomycin (Fig. 5), as well.

Unusual data were reported instead by Brown, who found combined vancomycin and daptomycin resistance in six isolates cultivated from an organ preservation fluid; such a finding clearly suggested differences in membrane composition of those organisms compared with susceptible strains described by other authors throughout the literature (Brown, 2012).

Hence, it is hard to foresee *B. cereus* behaviour under antibiotic exposure, and susceptibility testing is needed (case-by-case) in order to provide patients with adequate therapies. In general, however, it seems that susceptibility to clindamycin, erythromycin, chloramphenicol, ciprofloxacin, vancomycin, aminoglycosides, and tetracycline is frequent, today, and vancomycin, clindamycin, imipenem and aminoglycosides have been alternatively recommended as treatment options against severe *B. cereus* diseases (Katsuya, 2009; Strauss, 2001; Bottone, 2010); instead, broad-spectrum cephalosporins and ticarcillin-clavulanate should be avoided in the empirical treatment when an infection by this orgamism is suspected (Bottone, 2010).

However, it must be noted that serious infectious processes may require the use of a combined antibiotic therapy (Frankard, 2004).

Conclusion

The major hurdle in evaluating the clinical significance of *B. cereus* from a clinical specimen is overcoming its nagging stigmata as an “insignificant

contaminant", which is still in vogue in spite of an ongoing evidence of extraintestinal infections (Bottone, 2010; Dohmae, 2008).

Indeed, outside the notoriety of the organism in association with food poisoning, foodborne intoxications and ocular diseases, clinicians and microbiologists should both give serious consideration to the relevance of a clinical *B. cereus* isolate, especially if an underlying immune system impairment is present (Bottone, 2010).

B. cereus is not frequently responsible for pneumonia, but there are several reports of serious and often fatal cases (Table 1).

In this context, there is so far no conclusive explanation for the strong association between metalwork and *B. cereus* disease, although it is likely that professional exposure to a variety of environmental factors may place these subjects at additional risk of acquiring such infections (Hoffmaster, 2006; Bottone, 2010). In general, the literature suggests welders experience higher frequencies of pneumonias with enhanced severity and duration, while it is hard to link clinical cases with bacterial environmental sources (as *B. cereus* is known to be ubiquitous) (Hoffmaster, 2006). However, metalworkers are daily exposed to substantial quantities of dust contaminated by *B. cereus* spores; also, inhaling fine, respirable particles (including metal fumes) can subtly affect the immunologic defenses of lungs; therefore, acute airway infections in welders in general are more frequent, more severe, of longer duration and characterized by an increased mortality (Avashia, 2007).

Regarding compromised patients, instead, the invasion of the oral cavity in hosts with an underlying immune system impairment may occur as the oral cavity may become colonized with *B. cereus* either through the inhalation of spores or by vegetative bacteria; then, the organism can spread to adjacent tissues, or disseminate to other body sites through the bloodstream (Bottone, 2010).

In the pseudomembranous tracheobronchitis case cited, it is likely that treatment-mediated damage to the oral mucosa may have enhanced spore and vegetative cell adherence and colonization; in this context, it has been suggested that the establishment of *B. cereus* on the oral mucosa may well be an underappreciated initial stage in the pathogenesis of pulmonary as well as systemic infections in immunocompromised individuals (Strauss, 2001; Bottone, 2010).

Finaly, asthma and smoking might have behaved as additional predisposing factors in reported cases of *B. cereus* lung disease, although their role is poorly understood, currently (Avashia, 2007).

It is clear that *B. cereus*-related respiratory infectious conditions resemble those caused by *B. anthracis*; pulmonary anthrax is predominantly observed in livestock, and less commonly in humans (man anthrax is called the 'woolsorter's disease') (Forsberg, 2011; Miller, 2012). The infection is life-threatening, and generally accompanied by overwhelming septicemia; the clinical picture starts abruptly with high temperature, dyspnea, and chest pain; it progresses fastly and often brings patient to death before therapy can halt the invasive aspect of the infection (Miller, 2012). In this context, it is likely that in the published reports of anthrax-like *B. cereus* pneumonias the large numbers of *B. cereus* organisms together with the variety of enzymes and toxins they can produce could have led to the rapid course and unfavourable outcome of this pathology (Miller, 2012).

B. cereus spores, which are hydrophobic and have projecting appendages, adhere to small-intestine and colonic epithelial cells; *in vitro*, spores organize as aggregates that, when germinating, release high concentrations of tissue-destructive toxins (Bottone, 2010). Contact adherence of spores in clusters to epithelial cells may trigger their germination, as well as enterotoxin production, and disintegration of the epithelial tissue monolayer (Bottone, 2010). Interestingly, bacteria continue to adhere to membrane debris; likewise, the ingestion of spores with binding capability in the context of potentially disrupted airway mucosa could lead to cytotoxicity in the respiratory tract, as exemplified by the development of diphtheria-like membranes along with pulmonary infection and systemic dissemination (Bottone, 2010).

It may be of interest to note that the pseudomembranous tracheobronchitis case (Strauss, 2001) resembled diphtheria; in fact, formation of such a kind of structures in the respiratory tract is typically related to the *Corynebacterium diphteriae* infection, although pseudomembranous tracheobronchitis due to *Aspergillus* species and corynebacteria other than *C. diphteriae* have been reported in the recent years, in immunocompromised patients (Strauss, 2001).

To conclude, we may understand from the literature that current knowledge of the airway pathogenic potential of *B. cereus* still has to be deeply understood, and it is likely that further clinical and histopathologic pictures other than those described so far may be related to infection by this rod. Taken together, published works clearly demonstrate the need for an increased consciousness of this organism's pathogenicity for lung and the respiratory tract in general; hence, dismission of clinical isolates as contaminants (without a previous, careful evaluation) could lead to misdiagnosing and under-reporting.

B. cereus lung diseases have been observed both in immunocompetent and immunocompromised hosts (Table 1). Particularly, when subjects suffering from an immune system impairment develop chest pain and a productive cough with rapid worsening of the pulmonary function, an infection by this organism should be taken into account and included in the differential diagnosis (Strauss, 2001).

Recognition of this species airway tropism should lead to an early institution of antibiotic therapy and early surgical drainage, if required, when an infection is recognized (Carbone, 1985), in order to improve the outcome of such potentially life-threatening conditions.

Rapid diagnostic tests to discriminate virulent strains from the majority of *B. cereus* isolates are warranted (Avashia, 2007); also, a deeper awareness of public health significance of this pathogen is required, as well as an increased understanding of the occupational and environmental factors enhancing the susceptibility of individuals to *B. cereus* pulmonary disease.

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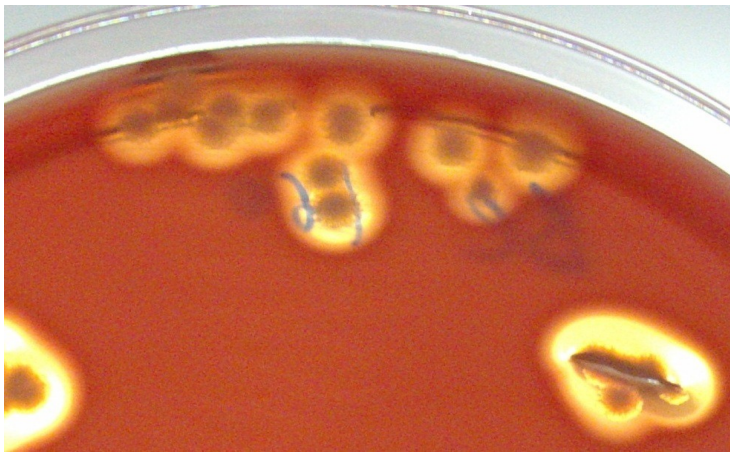

Fig. 1 – Hemolytic *B. cereus* strain (Bc160) from our laboratory collection after 24 h incubation (sheep blood agar by Liofilchem®, Roseto degli Abruzzi, Italy)

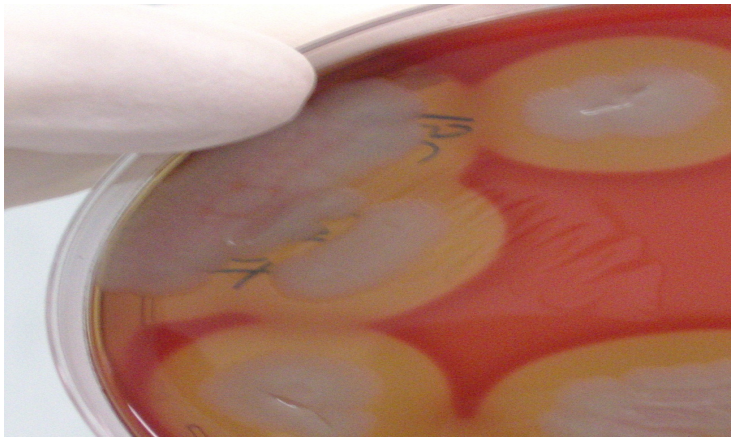

Fig. 2 – Bc160 colonies (see Fig. 1) after 48 h incubation (sheep blood agar by Liofilchem®, Roseto degli Abruzzi, Italy)

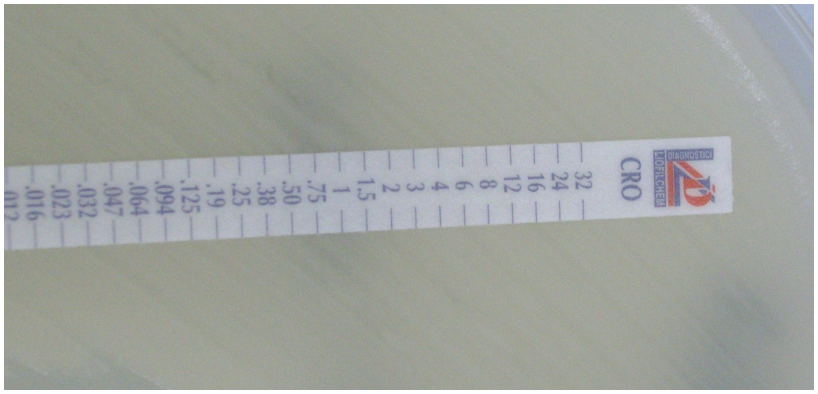

Fig. 3 – *B. cereus* (strain from our laboratory) high (>32 mg/L) ceftriaxone MIC (MIC Test Strip and Mueller-Hinton II agar by Liofilchem®, Roseto degli Abruzzi, Italy – CRO, ceftriaxone)

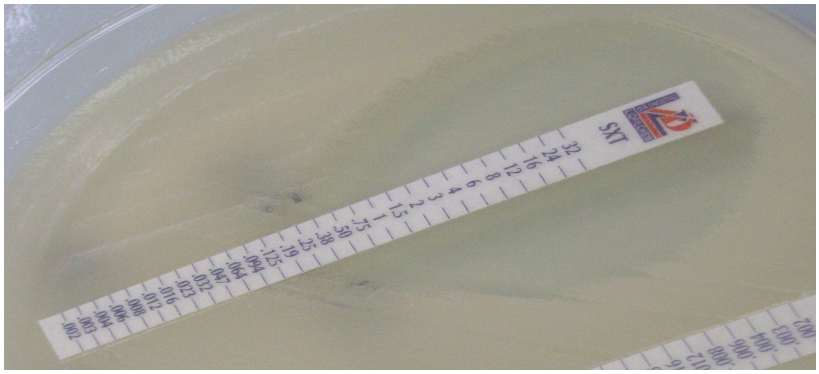

Fig. 4 – *B. cereus* (strain from our laboratory) low (0.19 mg/L) cotrimoxazole MIC (MIC Test Strip and Mueller-Hinton II agar by Liofilchem®, Roseto degli Abruzzi, Italy – SXT, cotrimoxazole)

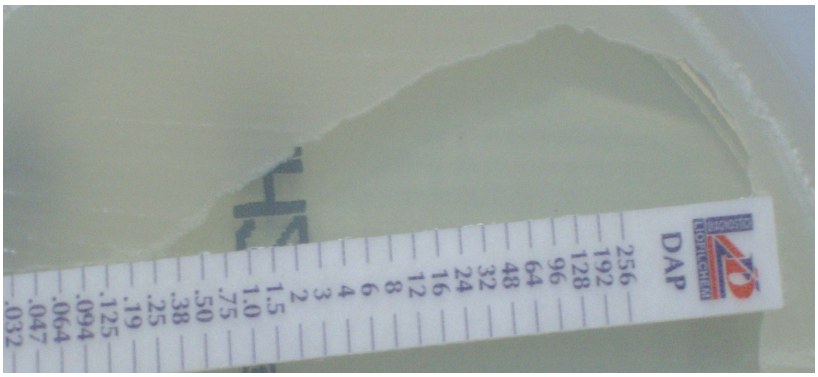

Fig. 5 – *B. cereus* (strain from our laboratory) low (0.25 mg/L) daptomycin MIC (MIC Test Strip and Mueller-Hinton II agar by Liofilchem®, Roseto degli Abruzzi, Italy – DAP, daptomycin)