بسم الله الرحمن الرحيم

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تم رفع هذه الرسالة بواسطة / سلوي محمود عقل

بقسم التوثيق الإلكتروني بمركز الشبكات وتكنولوجيا المعلومات دون أدنى مسئولية عن محتوى هذه الرسالة.

ملاحظات: لا يوجد

Introduction

Bacillus species is a gram-positive aerobic or facultative anaerobe were first identified by Christian Gottfried Ehrenberg in 1885 and were later classified by Ferdinand Cohn in to a separate genus Bacillus ***(Kandi, 2016).*** These spore-forming organisms, ubiquitously found in the natural environment at approximately 10^6 spores/g in soil, reportedly colonizes the human gastrointestinal tract at up to 10^4 spores/g of feces ***(Hong et al., 2009).***

Human infections by Bacillus spp. are infrequently reported in literature barring anthrax. The major drawback for the inadequate reporting of Bacillus spp. infections by clinical microbiology laboratories is the fact that most of these bacteria are saprophytic and their isolation in human clinical specimens is ignored as laboratory contaminants. Bacteraemia, endocarditis, wound infections, infections of the eyes and ears, respiratory tract infections, infections of the urinary and gastrointestinal tract, food poisoning and meningitis are a few clinical conditions where in Bacillus spp. have been isolated. Since not all human infections are caused among the immunocompetent individuals, Bacillus spp. other than B anthracis could well be recognized as

opportunistic pathogens in immunocompromized and debilitated individuals ***(Kandi, 2016).***

Among the many predisposing factors responsible for human infections with Bacillus spp. other than B anthracis, chronic alcoholism, presence of intravascular devices, intravenous drug abuse and trauma have been noted as significant ***(Tran & Ramarao, 2013).***

Human infections with Bacillus cereus have been frequently reported in literature and most infections were among debilitated patients including the hospitalized patients undergoing dialysis, Paediatric age patients and individuals suffering from haematological malignancies. Infection of B pumilus in an otherwise immunocompetent child should be considered as a cause for serious concern. Identification of gram positive aerobic spore forming bacilli to the species level, assessing the pathogenic potential of such bacteria and interpreting the antimicrobial susceptibility testing results against commonly used antibiotics is the need of the hour ***(Sharma & Rao, 2015).***

Laboratory identification of Bacillus spp. includes certain biochemical and physiological properties such as ability to grow anaerobically, motility, catalase production,

citrate utilization, nitrate reduction, fermentation of glucose ***(Kandi, 2016).***

A selective chromogenic media for identification and differentiation of B. cereus from other members of the group, these media contain synthetic fluorogenic and chromogenic substrates that are cleaved by specific enzymatic activities Incorporation of such substrates into selective media facilitates and improves the accuracy of detection and identification ***(Tewari et al., 2013).***

Matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS), which can be used to analyze the protein composition of a bacterial cell, has emerged as a new technology for species identification. MALDI-TOF MS is suitable for high-throughput and rapid diagnostics at low costs and can be considered an alternative for conventional biochemical and molecular identification systems in a conventional microbiological laboratory ***(Wieser et al., 2012).***MALDI-TOF MS method has been found to be useful in the rapid and reliable identification of vegetative cells of Bacillus anthracis, Bacillus cereus group as well as non-Bacillus cereus group ***(Lasch et al., 2008).***

In conclusion it must be understood that although Bacillus species other than B. Anthracis have been rarely associated with human infections, and that many clinical microbiology laboratories ignore these bacteria as laboratory contaminants, careful clinical and laboratory consideration is required to evaluate the actual role of these bacteria in causing human infections to effectively manage the patients ***(Kandi, 2016).***

Aim of the Work

The aim of the present thesis is to:

1- Isolation of pathogenic strains of bacillus species from different clinical samples from patient admitted in Ain Shams University Hospital.

2- Compare chromogenic media, Vite$^{k@}$2C ID cards and MALDI-TOF as gold standard method for identification of different bacillus species.

3- Determine minimal inhibitory concentration of different antibiotics by microbroth dilution method to make recommendation for Bacillus treatment.

Bacillus Species

Introduction:

Bacillus species are Gram-positive, spore-forming, rod-shaped, aerobic or facultative anaerobic bacteria. They were first identified by Christian Gottfried Ehrenberg in 1885 and were later classified by Ferdinand Cohn in to a separate genus Bacillus. The genus *Bacillus* belongs to the family *Bacillaceae* ***(Kandi, 2016).***

The genus Bacillus (B.) includes more than 300 species. Most of the bacillus species are saprophytes and only few members of the genus are responsible for accidental and opportunistic human infections ***(Xu and Cote, 2003).*** Apart from *B. anthracis*, the cause of concern nowadays is the increasing reports of human infections caused by non-anthrax *Bacillus spp*. like the *B. cereus*, *B. subtilis* and *B. licheniformis*, *B. alvei*, *B. brevis*, *B. circulans*, *B. coagulans*, *B. macerans*, *B. pumilus*, *B. sphaericus* and *B. thuringiensis.* Respiratory tract infections, bacteraemia, endocarditis, gastrointestinal tract infections, food poisoning, eye infections, meningitis, wound infections are a few clinical conditions wherein Bacillus spp have been implicated ***(Tuazon, 2016).*** The most important risk factors for

infections with non-anthrax bacillus include chronic alcoholism, presence of intravascular devices, intravenous drug abuse and trauma ***(Tran and Ramarao, 2013).***

Bacillus species are used in many medical, pharmaceutical, agricultural, and industrial processes that take advantage of their wide range of physiologic characteristics and their ability to produce a host of enzymes, antibiotics, and other metabolites. The bacitracin produced by *B. licheniformis* or *B. subtilis*, polymyxin by *B. polymyxa* and gramicidin by *B. brevis* are examples of antibiotics produced by *Bacillus spp.*. The spores of *B. stearothermophilus* are used to check heat sterilization procedures, and *B. subtilis subsp globigii*, which is resistant to heat, chemicals, and radiation, is widely used to validate other sterilization and fumigation procedures ***(Tuazon, 2016).*** Certain Bacillus species are important in the natural or artificial degradation of waste products. Some Bacillus insect pathogens are used as the active ingredients of insecticides. Because the spores of many Bacillus species are resistant to heat, radiation, disinfectants, and desiccation, they are difficult to eliminate from medical and pharmaceutical materials and are a frequent cause of contaminate on. They

are well known in the food industries as troublesome spoilage organisms ***(Gopal et al., 2015).***

Classification:

Bacillus spp. has been traditionally classified into different species based on its phenotypic characteristics, pathogenicity, clinical symptoms, host preference, and ecological niche ***(Rasko et al., 2005).*** However, with the development of rapid nucleic acid sequencing, the genus has been reorganized based on 16S ribosomal ribonucleic acid (rRNA) sequence analysis (Table 1). Only approximately 5% of the isolates are of clinical significance and are considered opportunistic pathogens ***(Dos santos PC, 2017).***

Table (1): Classification of *Bacillus species*

Genera and Species	
Bacillus Cereus Group	**Bacillus Circulans Group**
• Bacillus anthracis • Bacillus cereus (type species) • Bacillus mycoides • Bacillus megaterium • Bacillus thuringiensis • Bacillus cytotoxicus	• Bacillus circulans (type species) • Bacillus firmus • Bacillus lentus • Bacillus coagulans
Bacillus Subtilis Group	**Other Related Organisms**
• Bacillus licheniformis • Bacillus amyloliquefaciens • Bacillus subtilis (type species) • Bacillus pumilus	• Brevibacillus spp. • Paenibacillus spp**.**

(Tille, 2017)

Habitat:

(Hong et al., 2009) The carriage rate of *B. cereus* in stools of healthy asymptomatic individuals can reach up to 43% ***(Bottone, 2010)***.

Outbreaks due to B. cereus have been associated with the use of medical items such as oral medication, reusable ventilator air-flow sensors, contaminated alcohol prep pads, and contaminated hospital linens ***(Cheng et al., 2017).*** In food borne outbreaks, sources of *B. cereus* have been traced to rice, meat loaf, turkey loaf, mashed potatoes, beef stew, apples and hot chocolate sold in vending machines ***(Drobniewski, 1993).***

Epidemiologic studies on the microbiology of street heroin and injection paraphernalia demonstrated that Bacillus spp. as the predominant isolate from both specimens ***(Dancer et al., 2005).***

Diseases caused by bacillus species:

1) Diseases Caused by *Bacillus anthracis:*

Virulence factors and pathogenesis:

The pathogenicity of B. anthracis is governed by two large extra chromosomal plasmids namely pXO1 and pXO2. Plasmid pXO2 encodes for the proteins involved in capsule synthesis. The capsule is made up of poly-γ-D-glutamic acid, that inhibits the bacterial phagocytosis during infection and is weakly immunogenic in nature. On the other hand, the plasmid pXO1 encodes a three-component toxin that consists of 3 distinct proteins: protective antigen (PA) (82.7 kDa), lethal factor (LF) (90.2 kDa), edema factor (EF) (88.9 kDa) in addition to anthrax toxin activator A (AtxA), a central regulator for toxin synthesis. These secreted toxin units enable the pathogen to establish a systemic infection in short duration of time ***(Sharma et al., 2017; Ehling-Schulz et al., 2019).***

Host proteases in the blood and on the eukaryotic cell surface activate the PA by cutting-off a 20-kDa segment, exposing a binding site for LF and EF. The activated 63 kDa PA polypeptide binds to specific receptors on the host cell surface, thereby creating a secondary binding site for which

LF and EF compete. The complex (PA+LF or PA+EF) is internalized by endocytosis and, following acidification of the endosome, the LF or EF cross the membrane into the cytosol via PA-mediated ion-conductive channels. This is analogous to the A-B structure-function model of Cholera toxin with PA behaving as the B (binding) moiety (Fig.1). The EF, responsible for the characteristic edema of anthrax, is a calmodulin-dependent adenylate cyclase. (Calmodulin is the major intracellular calcium receptor in eukaryotic cells.) The only other known bacterial adenylate cyclase is produced by *Bordetella pertussis*, but the two toxins share only minor homologies ***(Finley, 2018).***

The LF appears to be a zinc-dependent metalloprotease that targets the members of MAP Kinase Kinase (MAPKK). The cleavage of N-terminus of MAPKK members results in decrease in the phosphorylation status of host heat shock protein 27 (hsp27), which is essential for maintaining the permeability across endothelial cell lining ***(Liu et al., 2012).*** The LT also cleaves the N-terminus of NOD-like receptor protein 1 (Nlrp1) resulting in the activation of caspase-1 enzyme in the host cell, which in turn causes N-terminus cleavage of pre-interleukin (IL) 1b and pre-IL18 ***(Chavarria-Smith and Vance, 2013).*** Moreover, the systemically

released LT reduces the phosphorylation level of translational proteins for examples eIF4B, eIF4E and rps6 leading to decreased translation of hypoxia-induced Factor-1a (HIF1a) which, in turn, results in to pathological conditions in the host like angiogenesis and metastasis ***(Ouyang et al., 2014).*** Finally, the LT is reported to cause modifications in the histone epigenetics of IL-8 promoter, which causes lesser binding of NF-jB transcription factor, hence causing reduced translational rates of IL-8 19 ***(Raymond et al., 2009).***

Another major virulence factor is anthrolysin O, which is one of the pore forming toxins that disrupts the membrane integrity of host cells by forming homogenous pores ***(Sharma et al., 2017).***

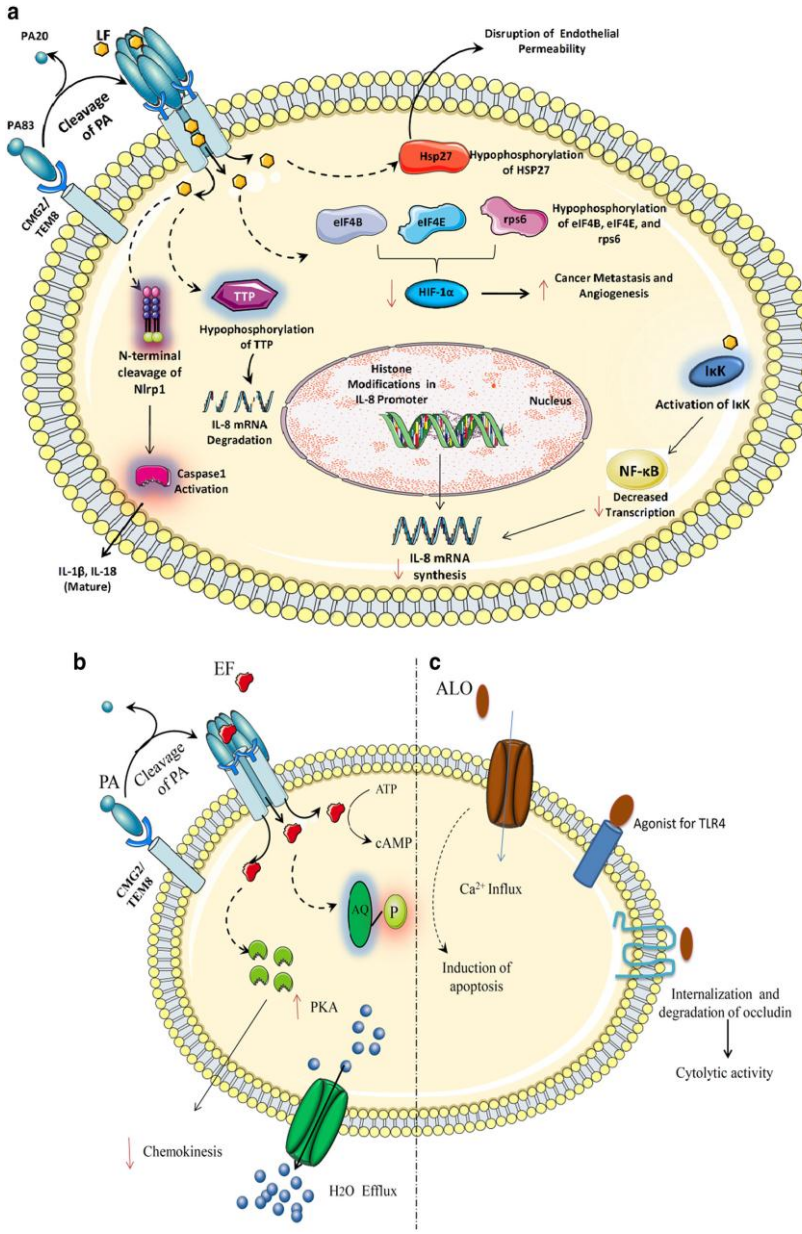

Figure (1): Mechanism of action of the anthrax toxin. Schematic diagram explaining the physiological imbalances observed in the host cells by anthrax major secreted virulence factors, which are lethal toxin (LT), edema toxin (ET), and anthrolysin O (AL) ***(Sharma et al., 2017).***

The toxin is composed of three proteins. Protective antigen (PA) binds to an appropriate site on the host cell membrane. A cell surface protease cleaves off a 20-kDa piece from the protective antigen and thereby exposes a secondary binding site for which lethal factor (LF) and edema factor (EF) compete. The complex (PA+LF or PA+EF) is internalized by receptor-mediated endocytosis, and acidification of the endosome results in the transfer of the LF or EF across the endosome membrane into the cytosol where they carry out their catalytic actions ***(Sharma et al., 2017).***

Clinical manifestation:

B. anthracis is the most virulent species of the genus Bacillus and is the causative agent of anthrax. Anthrax has afflicted humans throughout recorded history. The fifth and sixth plagues of Egypt described in Exodus are widely believed to have been anthrax. The disease was featured in the writings of Virgil in 25 BC and was familiar in medieval times as the Black Bane. It was from studies on anthrax that Koch established his famous postulates in 1876, and vaccines against anthrax the best known being that of Pasteur (1881)were among the first bacterial vaccines developed 20 ***(Sternbach, 2004).***