Prevalence of nanostructure of surface layer among the bacteria Isolated from environment

Shila Jalalpoor
Lecture of Microbiology, Shahreza Branch, Islamic Azad University, and Membership of young researchers club, Iran.
shilla.jalalpoor@yahoo.com, jalalpour@iaush.ac.ir

Abstract

Nanostructure of S-layer forms the monomolecular outermost protein layer in bacteria and archaea that credited with protein or glycoprotein subunits and has crystalline biopolymer structure. This layer protects bacteria to phagocytosis and prohibits the entry of some molecules e.g. antibiotics. The premises of hospital environment can serve as reservoirs of potential pathogens. The goal of study to was investigate the Nanostructure of Surface layer among the Bacillus cereus isolates from hospital environment. The research was performed with laboratory method during 2005-2007 from Azzahra Hospital and Esfahan University (Isfahan province of Iran). Totally, 194 samples were collected from hospital surface. Environmental samples collected with swab in Nutrient Broth (NB). Bacterial identification was performed based on Bacteriological standard methods using selective culture medium. Samples, cultured in TSA for 16 h, in aerobic condition were then separated for surface proteins and subjected to electrophoresis along with molecular weight marker. S-Layer in B. cereus has 97 KD of molecular weight. From 194 bacterial isolates among hospital environment, the frequency of Bacillus sp. was 26.29%. Among 13 B. cereus strains from hospital environment, only one strain (7.69%) was the producer of S-layer. Result showed that the prevalence B. cereus strain with S-layer in hospital sensitive environment is due to increased antibiotic resistance for nosocomial infection and it is necessary to continue the reduction of transfer of virulence agent and antibiotic resistant in pathogen bacteria.

Keywords: Nanostructure of Surface Layer, Bacillus cereus, Antibiotic Resistant, Hospital Environment

Introduction

Bacillus cereus bacteria are large spore forming, Gram-positive, rod-shaped and facultative anaerobic bacteria. B. cereus strains are common in the environment and can be found in soil, dust, air, water, and on decaying materials. It has been considered as a relatively nonpathogenic opportunistic commonly associated with enterotoxin mediated diarrheal food poisoning. This organism has been increasingly isolated from serious nongastrointestinal infections including endocarditis, wound infection, osteomyelitis, oral cavity associated with infected root canals, periodontal pockets, bovine mastitis, severe systemic, pyogenic infections, gangrene, septic meningitis, cellulitis, panophyhalmitis, lung abscesses, infant death, and endocarditis. Nowadays B. cereus regarded as one of the nosocomial infections bacteria (Amaout et al., 1999; Vander Zwet et al., 2000; Hilliard et al., 2003; Washington et al., 2006). Survival spore forming bacteria on hands and environment as vegetative cells can survive for at least 24 h, and spores survive for up to 5 months (Kamp & Kramer, 2004).

Nosocomial infections (NIs) remain a major global concern. Overall, national prevalence rates have been described as ranging between 3.5 and 9.9%. They lead to additional days of treatment, increase the risk of death, and increase treatment costs. Hospital environment have important role in NIs.

The health-care environment contains a diverse population of microorganisms. Environmental source or means of transmission of infectious agents, the presence of the pathogen does not establish its causal role; its transmission from source to host could be through indirect means, e.g., via hand transfer (Kim et al., 2000; Girard et al., 2002; Keith, 2005; Siegel & Chiarello, 2006; Agrawal et al., 2008). The surface would be considered as one of the potential reservoirs for the pathogen, but not the de facto source of exposure. An understanding of how infection occurs after exposure, one need to conclude that the chain of infection is also important in evaluating the contribution of the environment to healthcare-associated disease. All of the following components of the chain must be operational for infection to occur: 1. Adequate number of pathogenic organisms (dose); 2. Pathogenic organisms of sufficient virulence; 3. A susceptible host; 4. An appropriate mode of transmission of the organism in sufficient number from source to host; 5. The correct portal of entry into the host. Microbiologically contaminated surfaces can serve as reservoirs of potential pathogens (Kim et al., 2000; Girard et al., 2002; Keith, 2005, Agrawal et al., 2008; Siegel & Chiarello, 2006).

Over the past 3 decades of research, it has become apparent that one of the most common surface structures on bacteria are monomolecular crystalline arrays of proteinaceous subunits termed surface layer. Nano Structure of Surface layer is attached to the outermost proteinaceous subunits termed surface layer. Nano Structure of Surface layer is attached to the outermost portion of their cell wall. It consists of a single molecular layer composed of identical proteins or glycoproteins and in electron micrographs, has a pattern resembling floor tiles (Kotiranta et al., 1998; Sara, 2001; Mesnage et al., 2001; Messner et al., 2008). The Nano Structure of Surface layer lattices can have oblique (p1, p2) square (p4), or hexagonal (p3, p6) symmetry. Depending on the lattice type, one morphological unit consists of one, two, four, three, or six identical (glyco) protein subunits,
respectively, and they exhibit center-to-center spacing of approximately 2.5 to 35 nm. Most Nano Structure of Surface layer is 5 to 25 nm thick. It is now evident that Nano Structure of Surface layer are the most common cell surface components of pathogen bacteria such as *Lactobacillus* sp., *Rickettsia* sp., *Serratia* sp., *Caulobacter* sp., *Campylobacter* sp., *Corynebacterium*, *Clostridium* sp. and *Bacillus* sp. (Kotiranta et al., 1998; Sara, 2001; Mesnage et al., 2001; Messner et al., 2008).

The Nano Structure of Surface layer has been associated with a number of possible functions, these include the following: 1-The Nano Structure of Surface layer protect bacteria from harmful enzymes (Nano Structure of Surface layer from *Bacillaceae* were found to function as adhesion sites for cell-associated exoenzymes) and antimicrobial agents, 2-The Nano Structure of Surface layer protect bacteria from changes in pH, 3-The Nano Structure of Surface layer protect bacteria from attack by bacterial parasites such as *Bdellovibrio bacteriovorus*, and from bacteriophages, 4-The Nano Structure of Surface layer can function as an adhesion, enabling the bacterium to adhere to host cells and environmental surfaces, colonize, and resist flushing, 5-The Nano Structure of Surface layer may contribute to virulence by protecting the bacterium against complement attack and phagocytosis, and 6-The Nano Structure of Surface layer may act as a as a coarse molecular sieve. Nano Structure of Surface layer can contribute to virulence when they are present as a structural component of the cell envelope of pathogens (Sara, 2000; Schaffer & Messner, 2001; Eichler, 2003; Masahiro et al., 2003; Schaffer & Paul, 2005; Ghorbanzadeh et al., 2011a; Ghorbanzadeh et al., 2011b). This study presents the prevalence Nano structure of Surface layer among the *Bacillus cereus* isolated from hospital environment.

**Materials and methods**

**Sampling**

The research was performed with laboratory method during 2005-2007 years in Azzahra Hospital and Esfahan University. Overall study 194 sample from hospital environment. Hospital environment samples were randomly collected from high and low hospital contact surfaces with swab (Effective sampling of surfaces requires moistened swabs) in Tryptone Soya Agar (Merck) (Jalalpoor et al., 2007; Sehulster & Raymond, 2003).

**Bacterial strains**

Specimen grows on sheep blood and chocolate agars incubated at 37° C under aerobic conditions. Gram stains from blood cultures *Bacillus* as Gram-positive bacilli, intracellular and cell-free spores do not stain by the Gram technique but may be visualized with the malachite green stain, the spores will appear green. On SBA, colonies of *B.cereus* usually large, with a matte or granular texture, and most strains are beta hemolytic. The strains were identified based on colony morphology, Gram stain reaction, spore formation, and biochemical tests with the BioMerieux database system (Figure 1) (Washington et al., 2006; Jalalpoor et al., 2007).

**Detection Nano Structure of Surface layer**

For the examination of surface proteins, 16 h old bacterial cells cultured on TSA enriched with 0.6% yeast extract were collected from the agar plates, washed once in phosphate buffered saline (PBS) (pH 7.4), and suspended in the same buffer; the cell suspensions were adjusted to standard optic density; optical density of 0.6 (450 nm). Equal volumes (4 ml) of the cell suspensions were centrifuged (3,000 g in 6 min). The pellets were resuspended in 500 ml of 1% sodium dodecyl sulfate (SDS)-Tris-HCl (pH 8) and shaken for 30 min at RT. After centrifugation, the supernatants were boiled for 5 min in sample buffer (60 mM Tris-HCl, 1% SDS, 10% glycerol, 1% mercaptoethanol, and 0.0005% bromophenol blue) (Kotiranta et al., 1998, Kotiranta et al.,1999) and analyzed.

![Fig. 1. Bacillus cereus on Blood Agar (top) and Selective Bacillus cereus agar (bottom)](image1)

![Fig. 2. SDS PAGE of surface proteins in B.cereus strains](image2)
by SDS-10% polyacrylamide gel (PAGE) electrophoresis (Sambrook & Russel, 2001).

**Statistical analyses**

All the statistical analyses were carried out using SPSS version 14. Chi-Square and Fisher test used for determination of significance of association. The p < 0.05 was considered significant.

**Results**

Based on the results obtained from 194 samples, frequency of *Bacillus cereus* strains on hospital surfaces was 6.7%. Based on the results of SDS-PAGE, 1 (7.69%) of the studied bacteria (*B. cereus* strain) was the Nano Structure of Surface layer producer and 12 (92.31%) lacked the ability to produce Nano Structure of Surface layer (Fig.1). Fig. 2. shows the SDS PAGE of surface proteins in *B. cereus* strains. Fig. 3. represents the frequency Nano Structure of Surface Layer among the *B. cereus* strains isolated from hospital environment.

**Discussion**

- **Fig. 3. Frequency Nano Structure of Surface Layer among the Bacillus cereus strains isolated from hospital**

Based on the results obtained in this study, the frequency of *Bacillus cereus* strains on hospital surfaces was 6.7%. Earlier investigators also reported *Bacillus* spp. as abundant isolates of hospital surface (24%) (Jalalpoor et al., 2010a,b,c,d). Earlier studies on four strains of *B. cereus* isolated from clinical samples revealed that they could produce Nano Structure of Surface layer while the standard counterpart strains could not have produced Nano Structure of Surface layer (Kotiranta et al., 1998; Kotiranta et al., 1999).

Based on the results obtained in the present study, 1 (7.69%) of the strain isolated from hospital surfaces was the Nano Structure of Surface layer producer. Also, similar published studies indicate for the spread of *Bacillus cereus* strains resistant to antibiotics in hospitals. The lack of bacterial population control, leads to rapid release of antibiotic resistance from resistance strains to sensitive strains and ultimately leading to the spread of antibiotic resistance of nosocomial infections in hospitals and the community (Jalalpoor et al., 2009a,b; Jalalpoor et al., 2010a).

Approximately one third of nosocomial infections are preventable. Cleaning is the necessary first step of any sterilization or disinfection process. Cleaning may involve the removal of organic matter, salts, and visible soils, all of which interfere with microbial inactivation.

Environmental surfaces carry the least risk of disease transmission and can be safely decontaminated using less rigorous methods than those used on medical instruments and devices. Isolation precautions are designed to prevent transmission of microorganisms by common routes in hospitals. Because agent and host factors are more difficult to control, interruption of transfer of microorganisms is directed primarily at transmission (Sara & Uwe, 2000; Madani et al., 2009; Mielke, 2010).

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**References**