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Full Length Research Paper

Role of nano structure of crystalline layer and beta lactamase nano enzyme in antibiotics resistant bacteria

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Crystalline layer is the outermost protein in bacteria and archiae, and it inhibits antibiotics introduction due to increase of pathogenicity in bacteria. According to the role of health care workers and hospital surfaces in nosocomial infections, contamination of this source with *Bacillus cereus* strains produce crystalline layer and beta lactamase as a result of the spread of antibiotic resistance of nosocomial infections. The aim of this study is to survey the frequency of nano structure of crystalline layer and beta lactamase nano enzyme, and the role of crystalline layer in antibiotics inhibition in *B. cereus* strains isolated from health care workers and hospital surfaces. The research was performed with laboratory method in Azzahra hospital and Isfahan University. 274 samples were used for the preparation samples, which include culture bacteria in TSA (tryptone soya agar), separated surface proteins and specimen's electrophoresis with 10X SDS-PAGE. Samples antibiogram were performed with Kirby Bauer method, and beta lactamase production was detected with acidimetric method. Based on the result of SDS-PAGE, 46.20% of the studied. *B cereus* strains were able to produce crystalline layer and 53.8% were unable to produce crystalline layer. According to the antibiogram result, the non-producer strain of crystalline layer, when compared with the producer strain was sensitive to antibiotics and to all the strains that produced crystalline layer and beta lactamase. The result showed high prevalence of *B. cereus* strains that produced crystalline layer and beta lactamase in the hospital sensitive environment, due to increase of the antibiotic resistance of nosocomial infection. As such, it is necessary to reduce the transfer of virulence agent and antibiotic resistance in pathogen bacteria.

Key words: Crystalline layer nano structures, *Bacillus cereus*, resistant, Beta lactamase, nosocomial infections, virulence.

INTRODUCTION

All of the various surface components of a bacterial cell are important in its ecology since they mediate the contact of the bacterium with its environment. The only "senses" that a bacterium possesses result from its immediate contact with its environment. It must use its surface components to assess the environment and respond in a way that supports its own existence and survival in that environment. The surface properties of a bacterium are determined by the exact molecular composition of its membrane and cell envelope, including capsules, glycocalyx, crystalline layers, peptidoglycan and LPS, and the other surface structures such as, flagella and pili or fimbriae (Jalalpoor et al., 2007). 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. Health care worker and hospital surfaces have important role in NIs (Kampf and Kramer, 2004). The health-care environment contains a diverse population of microorganisms (Sehulster and Raymond, 2003: Jalalpoor et al., 2007). Microorganisms are present in great numbers in moist organic

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environments, but some also can persist under dry conditions. 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, via hand transferral (Sehulster and Raymond, 2003).

The surface would be considered as one of a number of potential reservoirs for the pathogen. Although, microbiologically contaminated surfaces can serve as reservoirs of potential pathogens, these surfaces generally are not directly associated with the transmission of infections to either staff or patients. The transferral of microorganisms from environmental surfaces to patients is largely via hand contact with the surface (Sehulster and Raymond, 2003). The most important and frequent mode of the transmission of nosocomial infections is divided into two subgroups: direct-contact transmission and indirect-contact transmission (Sehulster and Raymond, 2003).

Bacillus cereus bacteria are large spore forming Gram-positive rod-shaped, facultative anaerobes, and their strains are common in the environment and can be found in soil, dust, air, water, and on decaying substances. It has been regarded as a relatively nonpathogenic opportunist commonly associated with enterotoxin mediated diarrheal food poisoning. This organism has been increasingly isolated from serious non-gastrointestinal 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, and now *B. cereus* is regarded as one of the nosocomial infectious bacteria (Amaout et al., 1999, Hilliard et al., 2003, Van et al., 2000, Washington et al., 2006). Survival spore forming bacteria on hands and surfaces in vegetative cells can survive for at least 24 h on inanimate surfaces, and spores survive for up to 5 months (Kampf and Kramer, 2004).

Surface structures are important structural component of prokaryotic organisms and essential for many aspects of their life (Jalalpoor et al., 2007).

B. cereus produces several potential virulence factors in addition to the toxins associated with gastrointestinal infections, and these factors are thought to play a role in non gastrointestinal infections. These virulence factors include three hemolysins, three phospholipases, three different beta lactamases, extracellular collagenases, membrane-bound proteases and crystalline layer (Jalalpoor et al., 2007, Washington et al., 2006).

Nosocomial outbreaks of *Bacillus* infections have involved common-source spread from contaminated reservoirs in the environment. These sources have included contaminated hemodialyzers, bronchoscopes, Ommaya reservoirs, manual ventilation balloons, multiple-unit injectables, and contaminated diapers, gloves, and surgical bandages (Amaout et al., 1999, Jalalpoor et al., 2007, Hilliard et al., 2003, Van et al., 2000, Washington et al., 2006). In medical situations, the surface components of bacterial cells are major determinants of virulence for many pathogens. The surface properties of a bacterium are determined by the exact molecular composition of its membrane and cell envelope including capsules, glycocalyx, crystalline layer, peptidoglycan, LPS and the other surface structures such as, flagella and pili or fimbriae (Jalalpoor et al., 2007). 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 or crystalline layer. Crystalline 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 electron micrographs, has a pattern resembling floor tiles (Mesnage et al., 2001, Messner et al., 2008, Sara and Uwe, 2000; Sara, 2001; Schaffer and Messner, 2001). Because crystalline layer lattices possess pores identical in size and morphology in the 2 to 8 nm range, occupying up to 70% of the surface area, they work as precise molecular sieves, providing sharp cutoff levels for the bacterial cells. The repetitive features of crystalline layers have led to their use as immobilization matrices for binding of mono-layers of functional molecules for example; enzymes, antibodies, antibiotics and immunogens in a geometrically well-defined way. Crystalline layers can contribute to virulence when they are present as a structural component of the cell envelope of pathogens (Eichler, 2003; Masahiro et al., 2003; Sara and Uwe, 2000; Schaffer and Paul, 2005; Schaffer and Messner, 2001).

Beta lactam are antibiotics typically used to treat a broad spectrum of Gram-positive and Gram-negative bacteria. Beta lactamases produced by Gram-negative organisms are usually secreted. Beta lactamases are enzymes produced by some bacteria and are responsible for their resistance to beta lactam antibiotics, These antibiotics have a common element in their molecular structure; a four-atom ring is known as a beta-lactam. The lactamase enzyme breaks the ring open, deactivating the molecule's of antibacterial properties. Penicillinase is a specific type of beta lactamase, showing specificity for penicillins, again by hydrolysing the beta lactam ring. Penicillin and vancomycin are first selective and tetracycline and erythromycin is second selective antibiotic for therapy *Bacillus* sp. (Jalalpoor et al., 2007).

Spread of crystalline layer and beta lactamase produced *B. cereus* strains in health care workers and hospital surfaces due to increase of antibiotic resistant of NIs. According to the increase of antibiotic resistance, nosocomial infection in Iran and role of hospital surfaces and health care worker in nosocomial infection transfer bacteria in hospital, the aims of this research was to survey frequency of *Bacillus cereus* strains in hospital surfaces and health care workers, and survey the roles

of surface layer and beta lactamase in antibiotics resistant in *B. cereus* strains.

MATERIALS AND METHODS

Sampling

A total of 274 bacteria (194 bacteria from hospital surfaces and 80 bacteria from health care workers) were isolated from Azzahra-hospital from year 2006 to 2009. Hospital surfaces 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) (Sehulster and Raymond, 2003: Jalalpoor et al., 2007) and health care worker samples were randomly collected from health care worker in Blood Agar (Merck) via fingerprint technique (Sehulster and Raymond 2003, Jalalpoor et al., 2007).

Bacterial strains

Specimen grows on sheep blood incubated at 37°C under aerobic conditions. Gram stains from blood cultures *Bacillus* as Gram-positive bacilli, intracellular and cell-free spores were not stained by the Gram technique but may be visualized with the malachite green stain, the spores will appear green. On SBA, colonies of *B.cereus* are usually large, with a matte or granular texture, and most strains are beta hemolytic. The strains were identified on the basis of colony morphology, Gram stain reaction, spore formation, and biochemical tests with the BioMerieux database system (Kotiranta et al., 1998, 1999; Washington et al., 2006).

Detection of crystalline layer nano structure

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 a standard optical density of 0.6 (450 nm). Equal volumes (4 ml) of the cell suspensions were centrifuged (3,000 3 g, 6 min). The pellets were re-suspended in 500 ml of 1% sodium dodecyl sulfate (SDS)-Tris-HCl (pH 8) and shook 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.,1998,1999) and analyzed by SDS–10% polyacrylamide gel (PAGE) electrophoresis (Sambrook and Russell, 2001).

Detection of beta lactamase nano enzyme

Acidometric test is a diagnostic test for the rapid detection of the beta lactamase in bacteria (Jalalpoor et al., 2009a, 2007). Acidometric test is based on hydrolysis of the beta lactam ring, which resulted in the production of penicilloic acid. This process caused acidification of the bacterial suspension, and changed the color of the acidobasic indicator phenol red. The result of the reaction was very fast (Jalalpoor et al., 2009a, 2007). The red color of this indicator is present negative test and the yellow color of this indicator is present positive test (Figure 1) (Jalalpoor et al., 2009a, 2007).

Antibiotic susceptibility

Antibiotic susceptibility was performed according to antibiotic susceptibility standard disc diffusion agar (Wikler et al., 2009). The susceptibility of the isolates was determined against 4 antibacterial Penicillin, erythromycin, vancomycin and tetracycline.

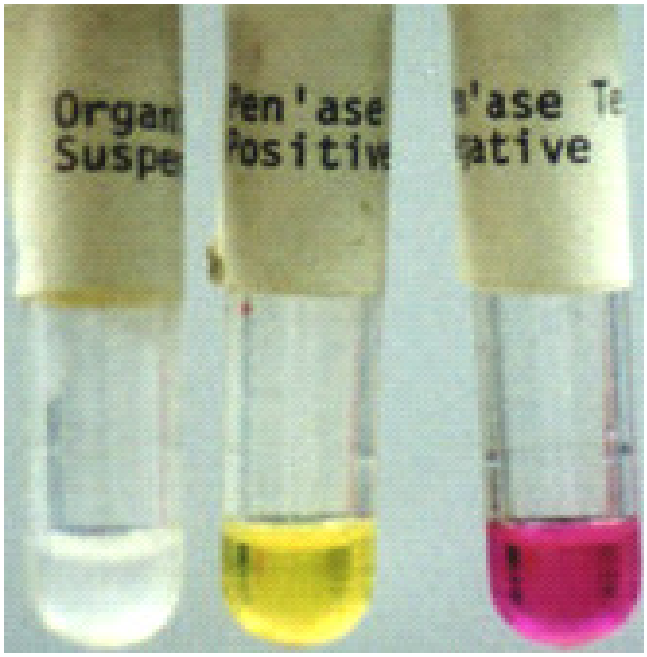

Figure 1. Beta lactamase production according to acidometric method.

Statistical analyses

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

RESULTS AND DISCUSSION

Based on the result obtained from 274 samples, frequency of *B. cereus* strain was 9.49%, thus the frequency of *B. cereus* in hospital surfaces and health care workers was 6.7 and 16.25%, respectively.

Based on the result of SDS-PAGE, 46.20% of the studied *B. cereus* strains produced crystalline layer and 53.8% were unable to produce crystalline layer. Thus, 84.6% of *B. cereus* strains isolated from health care workers and 7.7% of the strains isolated from the hospital surfaces produced crystalline layer (Figure 2). Based on the results of antibiogram, 100% of the *B. cereus* strains that produced crystalline layer were resistant to penicillin, 28% were resistant to erythromycin, 10% were resistant to vancomycin and 10% were resistant to tetracycline respectively (Figure 3). According to Acidometric test result, all of the *B. cereus* strains producing crystalline layer could produce beta lactamase, and only 7.10% of them could not produce beta lactamase and crystalline layer (Figure 4).

Based on the results obtained in this study, the frequency of *Bacillus cereus* strains on hospital surfaces

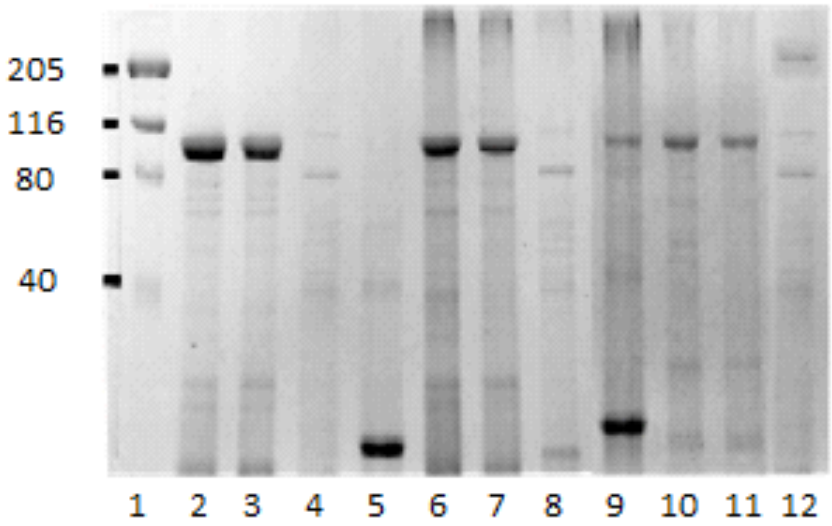

Figure 2. SDS PAGE of surface proteins in *B. cereus* strains. Lane 1, myosin 206 kDa, Beta-galactosidase 117 kDa, BSA 80 kDa, ovalbumin and 40 kDa; Lanes 2 to 12, *B. cereus* strains isolated from health care worker and hospital surfaces.

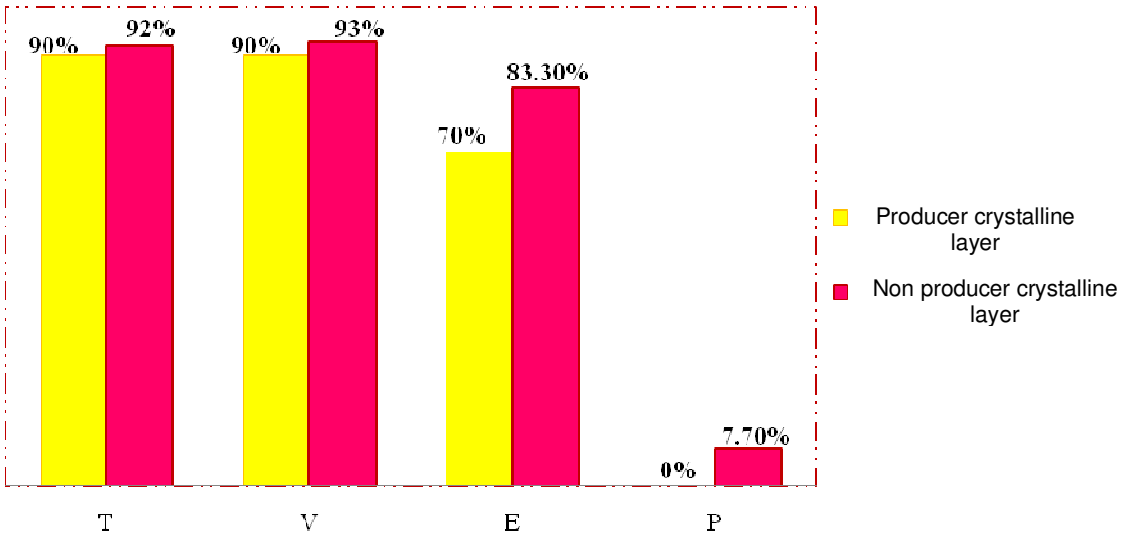

Figure 3. Sensitive pattern in tetracycline (T), vancomycin (V), erythromycin (E) and penicillin (P) in *B. cereus* strains producer and non producer crystalline layer.

and health care workers was 6.7 and 16.25%, respectively, whereas the results of other previously similar studies carried out in Iran showed that Bacillus species have been the major species used in bacterial separation from the hospital environment and health care workers; thus, the number of *Bacillus* spp. allocated to bacterial strains isolated from hospital surfaces was 74 (24%) and that from health care workers was 48 (60%) (Jalalpoor et al., 2009a, 2010c). Based on the results of similar studies in other countries, the frequency of Bacillus species in health care workers was 37% and frequency of *B. cereus* strains on health care worker has been reported as 15% (Amaout et al., 1999, Kampf and Kramer, 2004; Van et al., 2000).

Kotiranta et al. (1998, 1999) studied four strains of *B. cereus* and found out that strains isolated from clinical samples could produce crystalline layers, while standard strains could not produce crystalline layers.

Based on the results obtained from this study, 11 strains (84.60%) of *B. cereus* isolated from health care workers produced an S- crystalline layer, while only 1 strain (7.70%) isolated from hospital surfaces produced

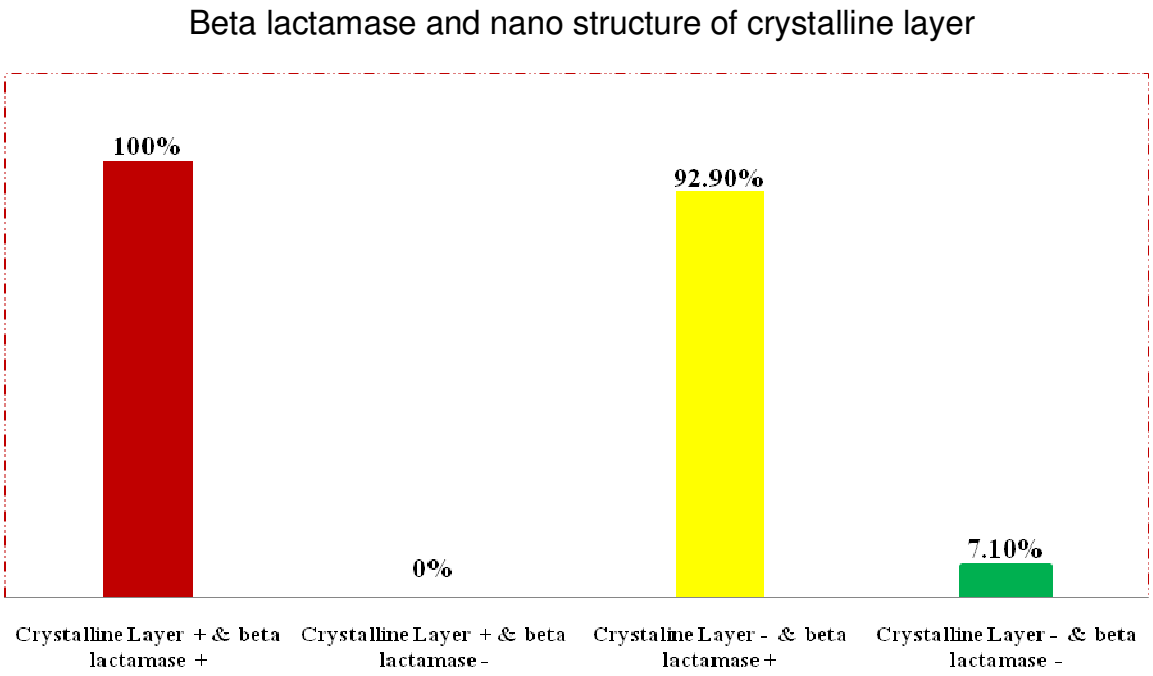

Figure 4. Frequency of crystalline layer and beta lactamase nano enzyme in *B. cereus* strains.

a crystalline layer.

The results of this study and other similar studies, treating many of crystalline layers in bacterial isolated from in vivo conditions, compared with bacterial isolated from in vitro conditions.

Regarding this point, *Bacillus cereus* is a human pathogenic bacteria and a crystalline layer structure is considered to be pathogenic, and can be interpreted in this case that the Bacillus cereus strains, if considered on biological conditions, produces crystalline layer to protect the influence of antibiotic and harmful enzymes in the human body (Jalalpoor et al., 2009b, c, 2010a)

Based on the antibiogram results, *Bacillus cereus* strains without crystalline layer compared with *Bacillus cereus* strains producer crystalline layer, was more sensitive to antibiotics. It is significant that 100% of *Bacillus cereus* strains produce crystalline layer and 92.3% of *Bacillus cereus* strains without crystalline layer, was resistant to penicillin. Based on these results, *Bacillus cereus* strains have been most sensitive against the antibiotic of vancomycin, tetracycline and erythromycin, respectively.

The results of this research and those of a similar published study indicated the spread of *Bacillus cereus* strains resistant to antibiotics in hospitals, and the lack of bacterial population control which leads to rapid release of antibiotic resistant genes from resistance strains in sensitive strains and ultimately lead to the spread of antibiotic resistance of nosocomial infections in hospitals and the community (Jalalpoor et al., 2010b, Jalalpoor and Hajiadineh 2011a, Jalalpoor and Abousaidi, 2011b, Jalalpoor, 2011c, d).

Conclusions

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 the interruption of the transfer of microorganisms, the transfer was directed primarily at transmission (Jalalpoor et al., 2009a, b, 2007; Mielke, 2010; Sehulster and Raymond, 2003). Approximately one third of nosocomial infections are preventable. Cleaning is the necessary first step of any sterilization or disinfection process. Cleaning is the removal of organic matter, salts and visible soils, all of which interfere with microbial inactivation; whereas hand washing is frequently the single most important measure used to reduce the risks of transmitting microorganisms from one person to another or from one site to another on the same patient. Although, hand hygiene is important to minimize the impact of this transfer, cleaning and disinfecting environmental surfaces appropriately is fundamental in reducing their potential contribution to the incidence of healthcare-associated infections (Jalalpoor et al., 2009a, b, 2007; Mielke, 2010; Sehulster and Raymond, 2003).

The results of this study and those of a similar study

indicated high frequency of crystalline layer in *Bacillus cereus* strains isolated from *in vivo* condition. Improvement of health care workers' hygiene and the control of bacterial population in hospital environment led to control of the transfer of antibiotic resistant genes from resistant strains in sensitive strains.

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