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Full Length Research Paper

Survey role of nano structure surface layer in *Bacillus cereus* strains resistant to antibiotics (Iran-Azzahra Hospital and Esfahan University)

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The most common surface structures bacteria are monomolecular crystalline arrays of proteinaceous subunits termed surface layers or S-layers. Since S-layer-carrying organisms are ubiquitous in the biosphere and because S-layers represent one of the most abundant cellular proteins, it is now obvious that these metabolically expensive products must provide the organisms with an advantage of selection in very different habitats. S-layers have been associated with a number of possible functions that relate to pathogenicity. S-layers can function as adhesins, enabling the bacterium to adhere to host cell membranes and tissue surfaces in order to colonize and protect bacteria from harmful enzymes and antimicrobial agents or changes in pH. *Bacillus cereus* is one of nosocomial infections bacteria. *B. cereus* produces several potential virulence factors such as S-layer. A total of 274 strains were isolated from staff hand and hospital surfaces of Azzahra-hospital during 2005 to 2007 years. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of S-layer proteins extracted from *B. cereus* strains by using Tris-HCl (pH 8) showed that the S-layer proteins of different strains isolate of staff hands and hospital surfaces had subunit molecular weight of 97-kDa. Antibiotic susceptibility was performed according to antibiotic susceptibility standard disc diffusion agar. All the statistical analyses 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. From 247 bacteria, frequency of *B. cereus* strains was 9.49%. From 13 isolated *B. cereus* of, staff hand 11 strain (84/6%) and from 13 isolated *B. cereus* from hospital surfaces, 1 strain (7/7%) production S-layer. According to the antibiogram result, S-layer non producer strains, in comparison with S-layer producer strains, were more sensitive to antibiotics. The result showed high prevalence of S-layer producer of *B. cereus* strains in hospital; and this point is due to the increased antibiotic resistance of nosocomial infections.

Key words: S-layer, *Bacillus cereus*, antibiotic resistant, nosocomial infections.

INTRODUCTION

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. Staff hands and hospital surfaces have important role in NIs (Jalalpoor et al., 2007; Kampf and Kramer, 2004). The health-care environment contains a diverse population of microorganisms (Sehulster and Raymond, 2003). Microorganisms are present in great numbers in moist, organic 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, e.g., through hand transferral (Sehulster and Raymond, 2003).

The surface would be considered one of a number of potential reservoirs for the pathogen, but not the de facto source of exposure. An understanding of how infection occurs after exposure, 4 based on the principles of the

chain of infection is also important in evaluating the contribution of the environment to health-care–associated disease. All of the 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 or transferal of the organism in sufficient number from source to host and (5) The correct portal of entry into the host. Although microbiologically contaminated surfaces can serve as reservoirs of potential pathogens, these surfaces generally are not directly associated with transmission of infections to either staff or patients. The transferral of microorganisms from environmental surfaces to patients is largely through hand contact with the surface (Jalalpoor, 2011; Sehulster and Raymond, 2003).

The most important and frequent mode of transmission of nosocomial infections, is divided into two subgroups: direct-contact transmission and indirect-contact transmission (Sehulster and Raymond, 2003).

Direct-contact transmission involves a direct body surface-to-body surface contact and physical transfer of microorganisms between a susceptible host and an infected or colonized person. Direct-contact transmission also can occur between two patients, with one serving as the source of the infectious microorganisms and the other as a susceptible host. Indirect-contact transmission involves contact of a susceptible host with a contaminated intermediate object, usually inanimate, such as contaminated instruments, needles, or dressings, or contaminated gloves that are not changed between patients and staff hands (Jalalpoor et al., 2007, 2011; Sehulster and Raymond, 2003).

Bacillus cereus bacteria are large spore forming, Gram-positive rod-shaped, facultative anaerobes. *B. cereus* strains are common in the environment and can be found in soil, dust, air, water, and on decaying. 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 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 and now *B. cereus* regarded one of nosocomial infections bacteria (Amaout et al., 1999; Eichler, 2003; Hilliard et al., 2003; Jalalpoor et al., 2010a, Van, 2000).

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 an 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 S-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 (Jalalpoor et al., 2007; Van et al., 2000; Washington et al., 2006).

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 (Jalalpoor et al., 2007). 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. 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, S-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 S-layer. S-layer 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 (Messner et al., 2008; Mesnage et al., 2001; Sara and Sleytr, 2000; Sara, 2001; Sleytr, 1997).

The S-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 spacings of approximately 2.5 to 35 nm. Most S-layers are 5 to 25 nm thick. It is now evident that S-layers 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. (Messner et al., 2008; Mesnage et al., 2001; Sara and Sleytr, 2000; Sara, 2001; Sleytr, 1997).

Because S-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. S-layers from various Bacillaceae were shown to be suitable for the production of isoporous ultrafiltration membranes with well-defined molecular weight cutoffs. The S-layer lattice and the pore areas of S-layers contain functional groups (carboxylic acid, amine, and hydroxyl groups) which are aligned in well-defined positions and orientations (Jalalpoor et al., 2007; Sara and Sleytr, 2000).

The repetitive features of S-layers have led to their use as immobilization matrices for binding of monolayers of functional molecules e.g., enzymes, antibodies, antibiotics and immunogens in a geometrically well-defined way. This application potential has been exploited for the production of bioanalytical sensors, immunoassays, affinity microparticles, and affinity membranes. The S-layer has been associated with a number of possible functions, these include the following: (1) The S-layer protect bacteria from harmful enzymes (S-layers from Bacillaceae were found to function as adhesion sites for cell-associated exoenzymes) and antimicrobial agents, (2) The S-layer protect bacteria from changes in pH, (3) The S-layer protect bacteria from attack by bacterial parasites such as *Bdellovibrio bacteriovorus*, and from bacteriophages, (4) The S-layer can function as an adhesin, enabling the bacterium to adhere to host cells and environmental surfaces, colonize, and resist flushing, (5) The S-layer may contribute to virulence by protecting the bacterium against complement attack and phagocytosis, and (6) The S-layer may act as a as a coarse molecular sieve. S-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 Sleytr, 2000; Schaffer and Messner, 2001; Schaffer, 2005).

Penicillin and vancomycin are first selective and tetracycline and erythromycin are second selective antibiotic for therapy *Bacillus* sp. (Jalalpoor et al., 2007). Spread of S-layer producer *B. cereus* strains in staff hand and hospital surfaces due to increase of antibiotic resistant NIs. According increase of antibiotic resistance nosocomial infection in Iran and role of hospital surfaces and staff hand in nosocomial infection and transfer bacteria in hospital, the aims of this search was survey frequency of *B. cereus* strains in hospital surfaces and staff hand and survey role of surface layer 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 staff hand were isolated of Azzahra-hospital during of 2005 to 2007 years. 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) (2,19) and staff hand samples, were randomly collected from staff hand in Blood Agar (Merck) through Fingerprint Technique (Jalalpoor et al., 2010a, c; Sehulster and 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 matter 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 S-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 phosphatebuffered 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, 3 g, 6 min). The pellets were resuspended in 500 ml of 1% sodium dodecyl sulfat (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 by SDS–10% polyacrylamide gel (PAGE) electrophoresis (Sambrook et al., 2001).

Antibiotic susceptibility

Antibiotic susceptibility was performed according to antibiotic susceptibility standard disc diffusion agar (Wikler et al., 2009).

Statistical analyses

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

RESULTS

Based on the results obtained from 274 sample, the frequency of *B. cereus* strains was 9.495, thus the frequency of *B. cereus* strains in hospital surface and staff hands was 6.7 and 16.25% respectively. Based on the results of SDS-PAGE, 46.20% of the studied *B. cereus* strains have been S-layer producer and 53.8% lack the ability to produce S-layer (Diagram 1). Thus the 84.6% of *B. cereus* strains isolated from staff hand and

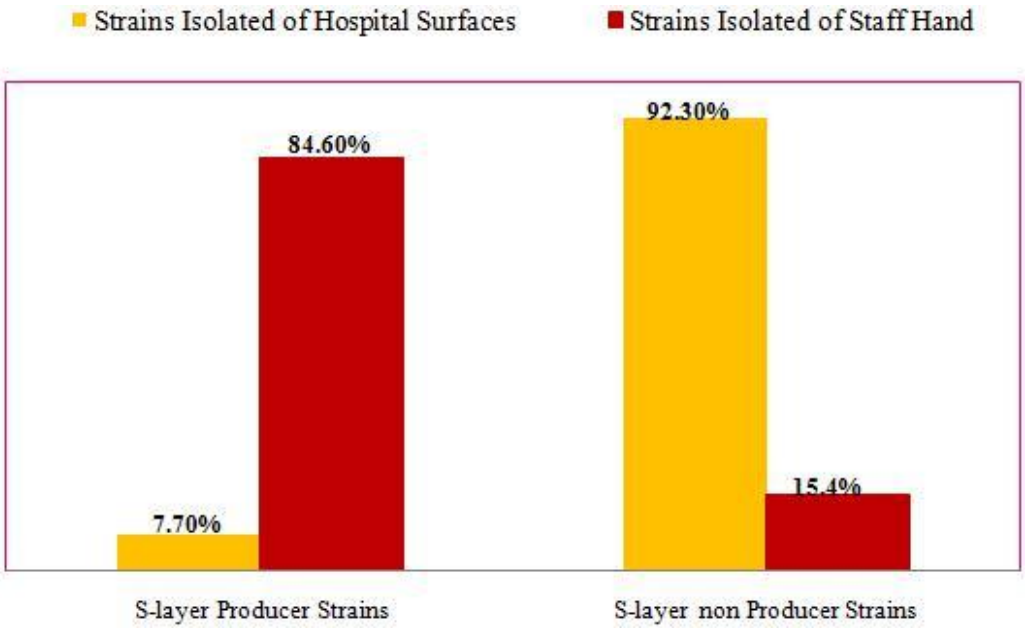

Diagram 1. Frequency of S-layer in *B. cereus* strains isolated from staff hand and hospital surfaces.

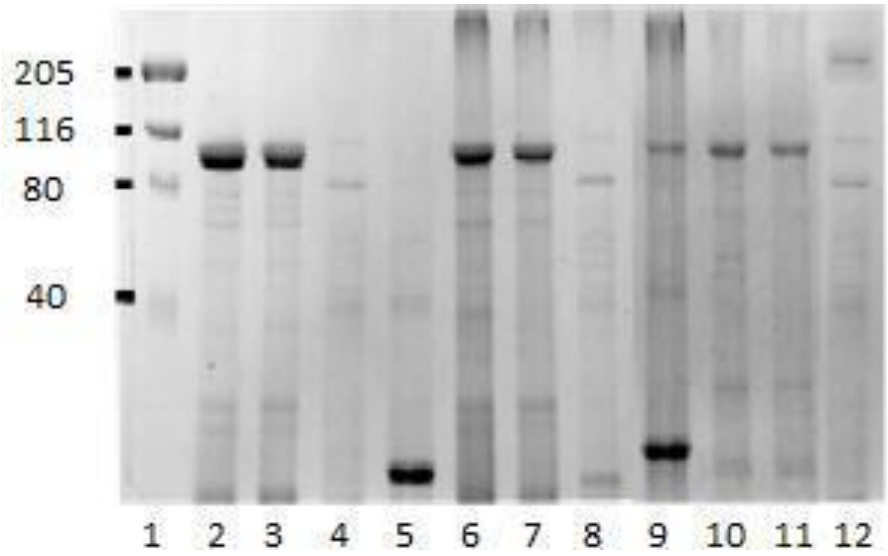

Figure 1. SDS PAGE of surface proteins in *B. cereus* strains. Lane 1: Myosin 206 kDa- Betagalactosidase 117 kDa- BSA 80 kDa- Ovalbumin, 40 kDa and Lane 2- 12 : *B. cereus* strains isolated from staff hand and hospital surfaces.

7.7% of the strains isolated from the hospitals surface have been S-layer producer (Figure 1).

Based on the results of antibiogram respectively 100% of S-layer producer *B. cereus* strains were resistance into penicillin, 28% of the S-layer producer *B. cereus* strains were resistance into erythromycin, 10% of S-layer producer *B. cereus* strains were resistance into vancomycin and 10% of S-layer producer *B. cereus* strains were resistance into tetracycline (Diagrams 2 to 5).

DISCUSSION

Based on the results obtained in this study, the frequency

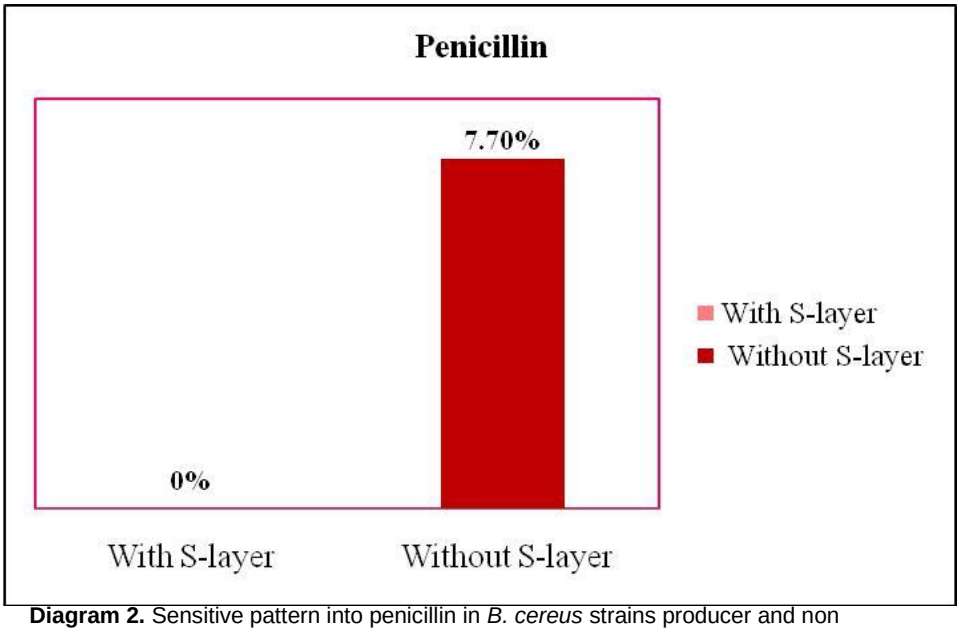

Diagram 2. Sensitive pattern into penicillin in *B. cereus* strains producer and non producer S-layer.

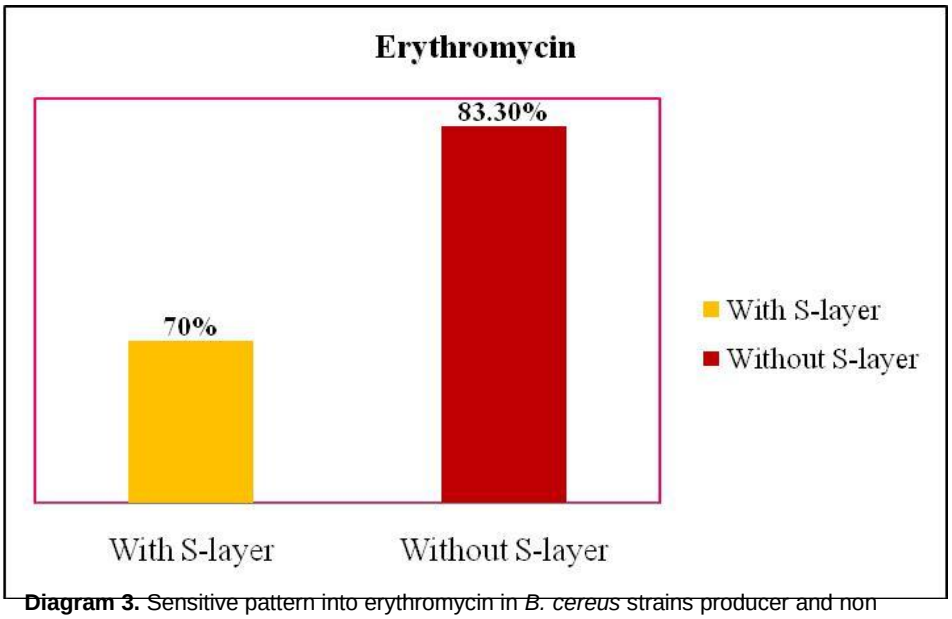

Diagram 3. Sensitive pattern into erythromycin in *B. cereus* strains producer and non producer S-layer.

of *B. cereus* strains on hospital surfaces and staff hand was 6.7 and 16.25% respectively, based on the results of other similar studies carried out in Iran, *Bacillus* species have been most bacterial separation from the hospital environment and staff hand, thus that *Bacillus* spp. were allocated from bacterial strains isolated of hospital surface 74 (24%) and from bacterial strains isolated of staff hands 48 (60%) (Jalalpoor et al., 2010a; 2009a; Wikler, 2009).

Based on the results of similar studies in other countries, the frequency of *Bacillus* species in staff hand was 37% and frequency of *B. cereus* strains on staff hand has been reported 15% (Amaout et al., 1999; Kampf and Kramer, 2004; Van et al., 2000).

Kotiranta et al. (1998, 1999) study on four strains of *B. cereus* and was determined, strains isolated from clinical samples can produce S-layer and standard strains could not have produce S-layer (Kotiranta et al., 1998, 1999).

Based on the results obtained in the present study, (11) 84.60% of *B. cereus* strains isolated from staff hand have been S-layer produce this while only (1) 7.70% of the strains isolated from hospital surfaces have been S-layer

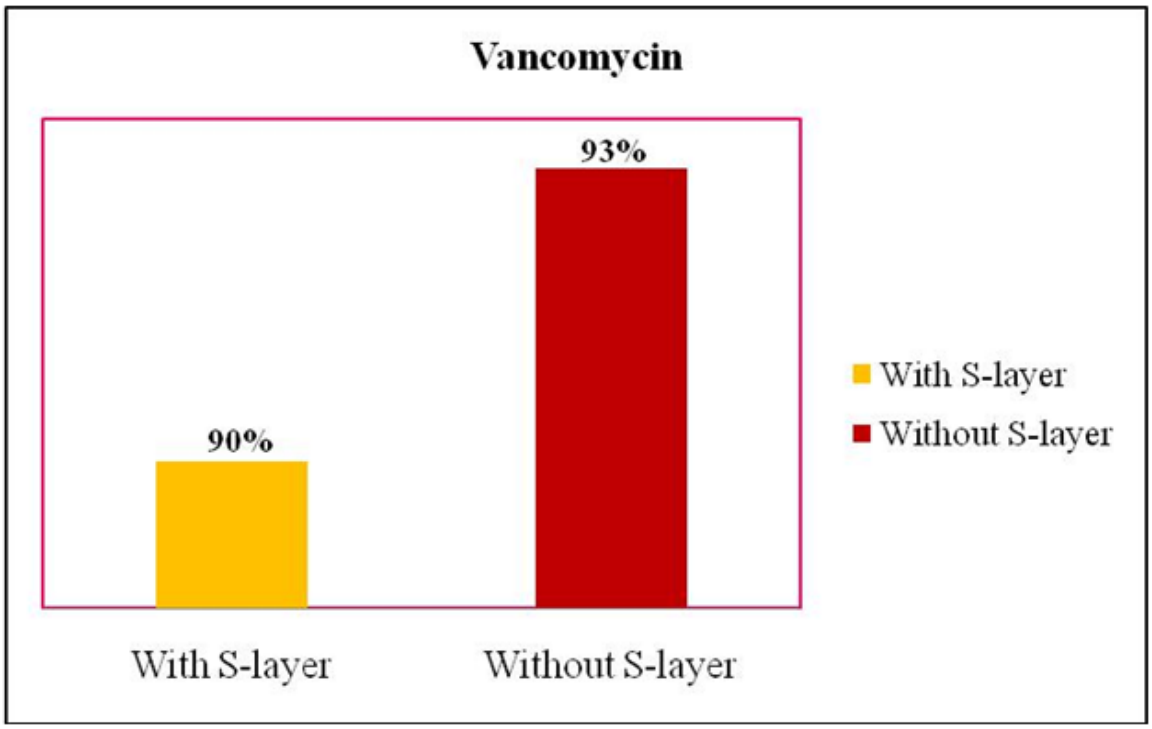

Diagram 4. Sensitive pattern into Vancomycin in B. cereus strains producer and non producer S-layer.

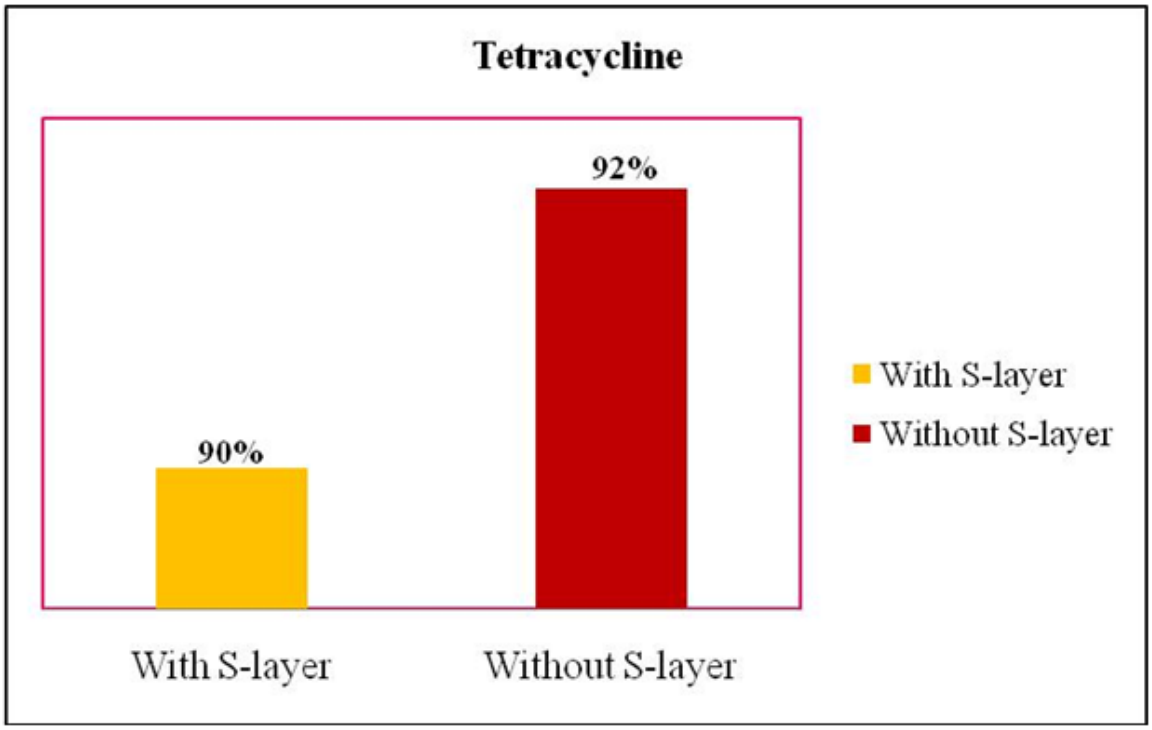

Diagram 5. Sensitive pattern into Tetracycline in B. cereus strains producer and non producer S-layer.

produce. The results of this study and other similar studies, treating many of S-layer in bacterial isolated from in vivo conditions, compared with bacterial isolated from in vitro conditions. Regarding this point B. cereus is a human pathogenic bacteria and a S-layer structure is considered to be pathogenic, can be interpreted in this case thought that the B. cereus strains, if considered on biological con-ditions, produces S-layer to protect

influence antibiotic and harmful enzymes in the body human (Jalalpoor et al., 2009b,c,d, 2010b,d,e). Based on the Antibiogram results, B. cereus strains without S-layer, compared with Bacillus cereus strains producer S-layer, was more sensitive into antibiotics. That is significant that 100% for B. cereus strains producer S-layer and 92.3% of B. cereus strains without S-layer, was resistant into penicillin. Based on these results, B. cereus strains have been the most sensitive against the antibiotic vancomycin,
tetracycline and erythromycin, respectively.

According to the results of this search and similar published study indicate spread of *B. cereus* strains resistant to antibiotics in hospitals, the lack of bacterial population control, leads to rapid release of antibiotic resistance genes from resistance strains in sensitive strains and ultimately leading to the spread of antibiotic resistance nosocomial infections in hospitals and the community (Jalalpoor et al., 2010c).

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, interruption of transfer of microorganisms is directed primarily at transmission.

Approximately one third of nosocomial infections are preventable. Cleaning is the necessary first step of any sterilization or disinfection process. Cleaning is removing organic matter, salts, and visible soils, all of which interfere with microbial inactivation. Hand washing frequently is called the single most important measure 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 as appropriate is fundamental in reducing their potential contribution to the incidence of healthcare-associated infections.

According to the results of the present study and those of a similar study, this study indicates high frequency of S-layer in *B. cereus* strains isolated from an *in vivo* condition. Improve of staff hand hygiene and control of bacterial population in hospital environment led to control of transfer of antibiotic resistance genes from resistance strains in sensitive strains.

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REFERENCES

Amaout MK, Tamburro RF, Bodner SM (1999). *Bacillus cereus* causing fulminanl sepsis and hemolysis in two patients with acule leukemia.Pediatr.Hematol.Oncol., 21: 431-435.

Eichler J (2003). Facing extremes: archaeal surface-layer (glyco) proteins. Microbiol., 149: 3347-3351.

Hilliard NJ, Schelonka RL, Waites KB (2003). *Bacillus cereus* bacteremia in a preterm neonate. J. Clin. Microbiol., 41: 3441-3444.

Jalalpoor Sh, Kasra Kermanshahi R, Noohi AS, Zarkesh H (2007).Study of -lactamase and S-layer Production in some of Isolated Pathogen Bacteria From Clinical and Environmental Hospital Samples. MSc thesis, Iran, Tehran, Islamic Azad University Science and Research Branch Tehran. Iran.

Jalalpoor Sh, Kasra Kermanshahi R, Nouhi AS, Zarkesh EH (2009a). The comparative frequency the β-lactamase Production and antibiotic susceptibility pattern of Bacterial stains isolated from Staff Hands and Hospital Surfaces in Azzahra Hospital–Isfahan. Iran. J. Med. Microbiol., 3(4): 37-45.

Jalalpoor Sh, Kasra Kermanshahi R, Nouhi AS, Zarkesh Isfahani H (2009b). The Prevalence of Nano-structure Surface Layer in *Bacillus cereus* Strains Isolated from Staff Hands and Hospital Surfaces. J, Isfahan. Med. School., 27(100): 632-645.

Jalalpoor Sh, Kasra Kermanshahi R, Nouhi AS, Zarkesh Isfahani H (2009c). Survey effect of in-vivo and *in-vitro* condition on expression of surface layer genes in bacteria. J. Iran. Chem. Soc., 6 (suppl): S11.

Jalalpoor SH, Abousaidi H (2009d). Survey Role and Important of Surfaces Structure and beta lactamase of *Bacillus cereus* in Drug Resistant. J. Microb. World, 2(3): 169-176.

Jalalpoor Sh, Kasra Kermanshahi R, Nouhi AS, Zarkesh Esfahani (2009e). Comparison of the Frequency β-lactamase Enzyme in Isolated Nosocomial Infectious Bacteria. J. Rafsanjan. Uni. Med. Sc., 8(3): 203-214.

Jalalpoor Sh, Kasra Kermanshahi R, Nouhi AS, Zarkesh Esfahani (2010a). Survey Frequence of β-lactamase Enzyme and Antibiotic Sensitivity Pattern in Isolated Pathogen Bacteria from Low and High Hospital Contact Surfaces. Pajuhandeh. J., 15(2): 77-82.

Jalalpoor Sh, Kasra Kermanshahi R, Nouhi AS ,Zarkesh Esfahani H (2010b). Survey Characterization Nano Structure Surface Layer in Some of Pathogen Bacteria. Zahedan. J. Res. Med. Sci., 12(4): 3-10.

Jalalpoor Sh, Kasra Kermanshahi R, Nouhi AS, Zarkesh Esfahani H, Sina Mobasherizadeh (2010c). Survey Prevalence and Resistance to Some Beta lactame Antibiotics in *Bacillus cereus* strains Isolated of AZZAHRA Hospital. Iran. J. Biol., 23(4): 470-477.

Jalalpoor Sh, Kasra Kermanshahi R, Nouhi AS, Zarkesh Esfahani (2010d). Prevalence of Nano Structure S-layer and β-lactamase in *Bacillus cereus* strains. J. Med. Sci. Islamic. Azad. Uni., 20(3): 157-163.

Jalalpoor Sh, Kasra Kermanshahi R, Nouhi AS, Zarkesh Esfahani H (2010e). The Role of nano structured surface layer and production of β–lactamase in penicillin resistant Bacillus cereus strains. Iran. J. Med. Microbiol., 4(1): 18-26.

Jalalpoor Sh (2011). Frequency of Beta Lactamase Enzyme in Isolated Pathogen Bacteria from Hospital *In-Vivo* and *In-Vitro* Condition. J. Isfahan. Med. Sch., 29(131): 1-9.

Kampf G, Kramer A (2004). Epidemiologic Background of Hand Hygiene and Evaluation of the Most Important Agents for Scrubs andRubs. Clin. Microbiol. Rev., 17(4): 863-893.

Kotiranta A, Haapasalo M, Kari K (1998). Surface Structure, Hydrophobicity, Phagocytosis, and Adherence to Matrix Proteins of *Bacillus cereus* Cells with and without the Crystalline Surface Protein Layer. Infect. Immun., 66(10): 4895-4902.

Kotiranta AK, Hitoshi I, Markus P, Haapasalo P, Kari L (1999). Radiation sensitivity of *Bacillus cereus* with and without a crystalline surface protein layer. FEMS. Microbiol. Lett., 179: 275-280.

Masahiro Y, Hirofuji T, Motooka N, Nozoe K, Shigenaga K, Anan H (2003). Humoral Immune Responses to S-layer-Like Proteins of *Bacteroides forsythus*. Clin. Diagnost. Laborate. Immune., 10(3): 383-387.

Messner P, Steiner K, Zarschler K, Schaffer C (2008). S-layer nanoglycobiology of bacteria. Carbohydr. Rese., 343(12): 1934-1951.

Mesnage S, Haustant M, Foue A (2001). A general strategy for identification of S-layer genes in the *Bacillus cereus* group: molecular characterization of such a gene in *Bacillus thuringiensis* subsp. galleriae NRRL 4045. J. Microbiol.,147: 1343-1351.

Sambrook J, Russell DW (2001). Molecular Cloning: A Laboratory Manual, 3rd Edition. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press.

Sara M, Sleytr UB (2000). S-layer Proteins. J. Bacteriol., 182(4):859-868.

Sara M (2001). Conserved anchoring mechanisms between crystalline cell surface S-layer proteins and secondary cell wall polymers in Gram-positive bacteria. Trends. Microbiol., 9: 47-49.

Schaffer C, Messner P (2001).Glycobiology of surface layer proteins. Biochimie, 83: 591-599.

Schaffer Ch, Paul M (2005).The structure of secondary cell wall polymers: how Gram-positive bacteria stick their cell walls together. Microbiology, 15: 643-651.

Sehulster L, Raymond YW (2003). Guidelines for Environmental Infection Control in Health-Care Facilities. U.S. Department of Health and Human Services Centers for Disease Control and Prevention (CDC). Atlanta GA 30333.

Sleytr UB, Bayley H, Sara M, Breitwieser A , Kupcu S, Mader CH, Weigert S (1997).Applications of S-layers. FEMS. Microbiol. Rev., 20: 151-175.

Van der Zwet WC, Parlevliet GA, Savelkoul PH, Stoof J, Kaiser AM (2000). Outbreak of *Bacillus cereus* infection in a neonatal intensive care unit traced to balloons used in manual ventilation. J. Clin. Microbiol., 38: 4131-4136.

Washington C, Stephen A, Janda W, Koneman E, Procop G ,Schreckenberger P, Woods G (2006). Koneman's Color Atlas and Textbook of Diagnostic Microbiology, Sixth edition.USA: Lippincott wiiliams & wilkins, pp. 775-779.

Wikler MA, Cockerill FR, Craig WA, Dudley MN, Eliopoulos GM, Hecht DW (2009). Performance standards for Antimicrobial Susceptibility Testing, Clin. Lab. Standards Inst., 29(3): 32-44.