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Full Length Research Paper

Survey beta lactamase production and resistance pattern into beta lactame antibiotics in *Bacillus cereus* strain isolated from staff hands and hospital environment in Iran

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Hospital infection is one of the important agent morbidity and mortality in patient. *Bacillus cereus* is one of the bacteria that cause nosocomial infection. Hospital surfaces and staff hands have important to creation nosocomial infections. Hospital surfaces have potentional capacity to carry bacteria and staff hands are the most reason for the transmission of bacteria to hospital. The present study was performed at one tertiary care hospitals in Isfahan, Iran. During a 30 month period. According to statistical formula, randomly selected 328 samples were needed for study, that from this samples 164 samples was environmental and 164 samples was clinical. Environmental and staff hands samples respectively were randomly selected from different ward of hospital and fingerprint technique, then samples cultured on Blood agar and EMB and were examined by Kirby Bauer Antibiogram and Acidimetric test. Of 328 samples, 21 samples was *B. cereus*. According to the result the prevalence of *B. cereus* was 6.4% (p=0.56) so, antibiotic resistance in *B. cereus* strain for Penicillin, Cefotaxime and Ampicillin were respectively 81, 47.6 and 19%. According to Acidimetric test 100% of isolated *B. cereus* strains from environmental, and 92.30% from staff hands product β–Lactamase. Result demonstrate high prevalence of resistance to β–Lactamase antibiotics *B. cereus* strain. One of the reason to create antibiotic resistant in bacteria is to increase contact of Bacteria.

Key words: *Bacillus cereus*, β–lactamase, antibiotic resistance, nosocomial infection, staff hands, hospital surfaces.

INTRODUCTION

The genus *Bacillus* comprised of a large group of aerobic and facultative, catalase positive, Gram-positive rods that are characterized by the ability to form spores under aerobic conditions (Jalalpoor et al., 2007; Washington et al., 2006). Members of the genus *Bacillus* that are recovered from clinical specimens usually grow well and sporulate on sheep blood (SBA) and chocolate agars incubated at 37°C under aerobic conditions. On SBA, colonies of *Bacillus cereus* are usually large, with a matte or granular texture, and most (Figure 1). Sporulation may be stimulated by subculture onto nutrient agar supplemented with $MnSO_4$ (5 µg/ml final concentration) and subsequent incubation. On Gram stains from blood cultures, other broth media, and occasionally solid media, *Bacillus* and related species may appear as Gram-negative bacilli, resulting in the mistaken impression that the organism is a Gram-negative, non fermentative bacillus. Inoculation of broth media and preparation of a Gram stained smear after a few hours of growth may reveal the Gram-positive nature of the organisms (Jalalpoor et al., 2007; Washington et al., 2006) (Figure 2). Intracellular and cell-free spores do not stain by the Gram technique but may be visualized with the malachite green stain. A smear of the organism is made on a slide, heat-fixed, and stained with 10% malachite green for 45 min. The smear is washed, counterstained with safranin for 30 s, and observed under oil immersion. The spores will appear green, while vegetative cells are stained pink

Figure 1. Separated staff hands specimens with fingerprint technique.

Figure 2. *B. cereus* on a plate of blood agar and *B. cereus* selective base agar.

Figure 3. β-Lactamase production with acidimetric method.

to red (Jalalpoor et al., 2007; Washington et al., 2006) (Figure 3). *B. cereus* has been regarded as a relatively nonpathogenic opportunist commonly associated with enterotoxin mediated diarrheal food poisoning (Beecher et al., 1995; Blaser et al., 1993; Beveridge, 1997). *B. cereus* strains produce one emetic toxin and three enterotoxin (Granum et al., 1997; Washington et al., 2006). This organism has been increasingly isolated from serious nongastrointestinal infections including endocarditis, wound infection, and osteomyelitis meningitis. Recently *B. cereus* has been found in the oral cavity associated with infected root canals and periodontal pockets (Barrie et al., 1994; Jalalpoor et al., 2009a; Kotiranta et al., 1998; Kotiranta et al., 1999; Schricker et al., 1994).

Serious *Bacillus* species infections have been associated with operative procedures, immunosuppres-sion, traumatic wounds, burns, hemodialysis, and potential drug abuse (Washington et al., 2006). *B. cereus* nosocomial infections classification in two groups: (1) gastroenteritis (2) non gastroenteritis infections.

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 (Van der Zwet et al., 2000). Bacteria strains, which have virulence factors, are more pathogenic to other strains. One of the virulence factors is antibiotic resistance. Stress of anti bacterial agents (e.g., disinfectants, antibiotics…). According to the importance of staff relationship, hand contamination in hospital surfaces in spreading bacteria in a hospital and the creative nosocomial infections, should continually be paid attention to when studying this agent. Nosocomial infection, also called “hospital acquired infection” can be defined as: an infection acquired in a hospital by a patient who was admitted for a reason other than that of infection (Jalalpoor et al., 2009b). An infection occurring in a patient in a hospital or other health care facility in whom the infection was not present or incubating at the time of admission. This includes infections acquired in the hospital but appearing after discharge, and also occupational infections among staff of the facility, nosocomial infections occur worldwide and affect both developed and resource-poor countries. Infections acquired in health care settings are among the major causes of death and increased morbidity among hospitalized patients. They are a significant burden both for the patient and for public health. A prevalence survey conducted under the auspices of WHO in 55 hospitals of 14 countries representing 4 WHO Regions (Europe, Eastern Mediterranean, South-East Asia and Western Pacific) showed an average of 8.7% of hospital patients had Nosocomial infections, at any time, over 1.4 million people worldwide suffer from infectious complications acquired in hospital , The highest frequencies of Nosocomial infections were reported from hospitals in the Hospital-acquired infections add to functional disability and emotional stress of the patient and may, in some cases, lead to disabling conditions that reduce the quality of life, Nosocomial infections are also one of the leading causes of death. The economic costs are considerable. The increased length of stay for infected patients is the greatest contributor to cost (Ducel et al., 2002; Jalalpoor et al., 2009b; Raymond et al., 2000). Normal human skin is colonized with bacteria, different areas of the body have varied aerobic bacteria counts (e.g., 1×10^6 colony forming unites (CFUs)/cm^2 on the scalp, 5×10^5 CFU/cm^2 on the axial, 4×10^4 on the abdomen and 1×10^4 on the forearm). Total bacteria counts on the hands of medical personnel have ranged from 3.9×10^4 to 4.6×10^6 (Boyce et al., 2002; Jalalpoor et al., 2009b). In overall bacteria on the hands were divided into two groups: (1) Transient bacteria, (2) Resident bacteria.

Transient flora which colonize the superficial layers of the skin, are more amenable to removal by routine hand washing. They are often acquired by health-care worker, (HCWs) during direct contact with patients or contact with contaminated environmental surfaces. Transient flora, are the organisms most frequently associated with health-care associated infections (Boyce et al., 2002). Resident flora, which is attached to deep layers of the skin, are more resistant to removal. In addition, resident flora (e.g. coagulase-negative staphylococci and diphtheroids) are less likely to be associated with hospital infections (Boyce et al., 2002). Transfer of bacteria in hospital environment, performed in three methods:

(1) Directly, from patient to patient.
(2) In directly, by transient carriage on the hands of HCWs.
(3) By hand transfer of organisms from contaminated environmental surfaces and patient are equipment (CDC, 2003).

The health-care environment contains a diverse population of microorganisms, microorganisms are present in great number in moist and organic environments, but some also can persist under dry conditions. The surface is one of a number of potential reservoirs for the pathogen, but they do not establish its causal rule; it's transmission from source to host could be through indirect means: (e.g. through hand transferal) (CDC, 2003).

Spread of infecting organisms from patient to patient and hospital surfaces, is usually done by the nurses, doctors and others caring for the patient. The major method of spread is on contaminated hands. Over a hundred years after Semmelweiss we still have not learnt the lesson he taught. Semmelweiss reduced mortality rates substantially in his hospital by insisting that students wash their hands before moving from the autopsy room to the maternity wards, and was consesquently hounded from office and died (ironically, of septicaemia) in a mental institution (Jalalpoor et al., 2009b). Antimicrobial resistance is an ever-increasing problem in all of hospitals, in world, especially in intensive care units (Jalalpoor et al., 2009b). Antibiotic resistance is the ability of a micro-organism to withstand the effects of an antibiotic. It is a specific type of drug resistance. Antibiotic resistance evolves naturally through natural selection through random mutation, but it could also be engineered. Once such a gene is generated, bacteria can then transfer the genetic information in a horizontal fashion (between individuals) by plasmid exchange. If a bacterium carries several resistance genes, it is called multiresistant or, informally, a superbug. The antibiotic action is an environmental pressure; those bacteria which have a mutation allowing them to survive will live on to reproduce. They will then pass this trait to their offspring, which will be a fully resistant generation. Break down the antibiotic with enzymes is an important method of resistance for many bacteria. β-Lactams are for example broken down by β-Lactamases. There is a whole host of β-Lactamases, and new ones seem to be discovered every day (Jalalpoor et al., 2009c). β-Lactamases is a type of enzyme produced by some bacteria that is responsible for their resistance to β-Lactam antibiotics like penicillin's, cephalosporin's, cephamycins and carbapenems. These antibiotics have a common element in their molecular structure: a four-atom ring known as a β-Lactam. The lactamase enzyme breaks that ring open, deactivating the molecule's antibacterial properties. Penicillinase is a particular type of β-Lactamase, showing specificity for penicillin's, again by hydrolysing the β-Lactam ring. Molecular weights of the various penicillinases tend to cluster near 50,000. Penicillinase was the first β-lactamase to be identified: it was first isolated by Abraham and Chain in 1940 from *Escherichia coli* even before penicillin entered clinical use (Jalalpoor et al., 2009c). Subject of this paper was survey β-Lactamase production and resistance pattern into some of β-Lactame antibiotics in *Bacillus cereus* strain isolated from staff hands and hospital surfaces in Azzahra hospital in Isfahan.

MATERIALS AND METHODS

Isolation and preparation of hospital surfaces samples performed

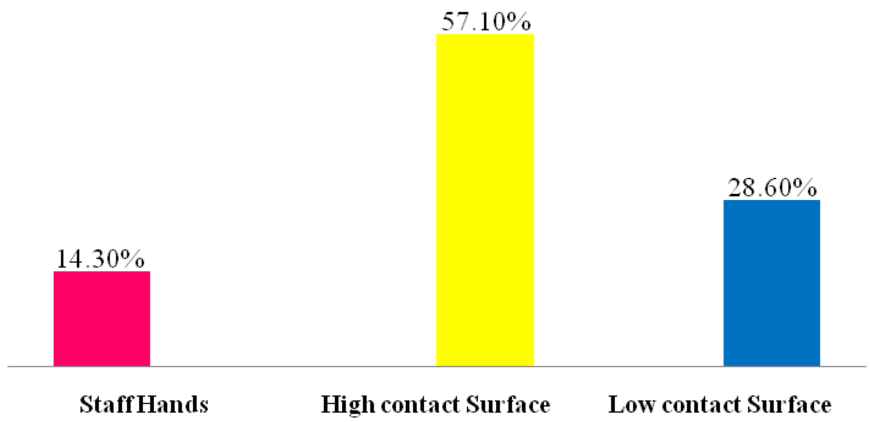

Diagram 1. Frequency of *B. cereus* according to source of separated.

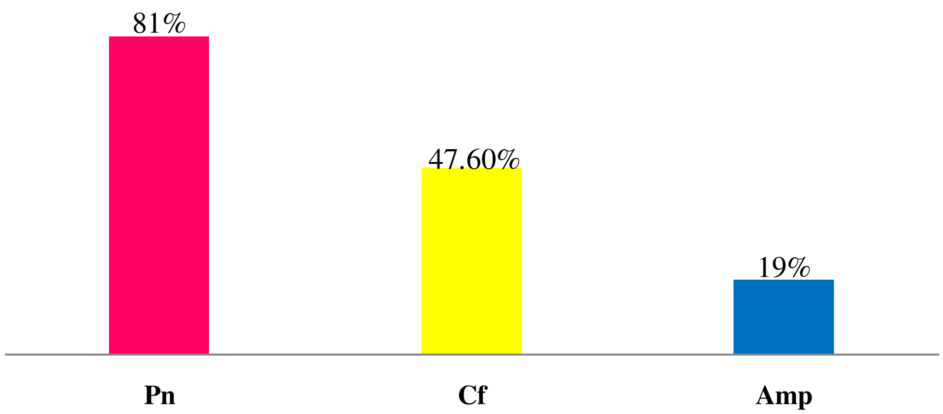

Diagram 2. Frequency of β-Lactame antibiotics resistance in *B. cereus* strains.

with moistened swabs in Nutrient broth (NB) then samples transfer to laboratory and cultured according to streak plate method on Blood agar and EMB, in aseptic condition (CDC, 2003; Jalalpoor et al., 2007).

Samples were collected from high contact surface (door knobs, bedrails and windows) and low contact surface (floors, ceilings) in room of patient in different ward of hospital (e.g. CCU, ICU, laboratory (Bond et al., 2004; CDC, 2003; Jalalpoor et al., 2007). Staff hands sampled was collected according to fingerprint technique on Blood agar and EMB (201) (Figure 1).

Identification of Bacteria, were performed with microbiological methods: staining, chemical test, use of differential and selective media (Jalalpoor et al., 2009b,c; Washington et al., 2006). Isolated *B. cereus* strains, performed with *B. cereus* selective agar (Figure 2) and for determine β-Lactamase production, use Acidimetric method (Figure 3). For determine, resistant pattern into β-Lactame antibiotics (Ampicillin, Penicillin and Cefotaxime) bacteria were antibiogram with Kirby-Bauer method (Jalalpoor et al., 2007; Washington et al., 2006; Wikler et al., 2006).

RESULTS

In this research, from 328 samples collected, that separated from different wards of hospital (environmental samples) and hands of personnel (clinical samples), totally isolated 21 *B. cereus* strain, that represent frequency of *B. cereus* stains were 6.4%. Frequency of these bacteria in environmental and clinical samples was 6.1 and 6.7%, respectively. According to result difference between two group, were no statistically significant ($p=0.56$).

In that manner showed 57.1% of isolated this bacteria, were from high contact surface in hospital and 14.3% were isolated from hands of personnel. According to result difference between two group, were statistically significant ($p=0.005$) (Diagram 1). Result of antibiotic susceptibility showed 81% of *B. cereus* strain was resistance to Penicillin (Pn), 47.6% to Cefotaxime (Cf) and 19% to Ampicillin (Am) (Diagram 2). On the base of results, the most of β-Lactame antibiotic resistance in *B. cereus* strain were observed to Pn. According to acidometric test 96/15% of strains product β-Lactamase and 100% of isolated *B. cereus* from environmental and 92/30% of isolated *B. cereus* from staff hands can product β-Lactamase.

DISCUSSION

Comparing the results obtained in this research with the results obtained in previously published articles that are similar to this research. Results of Acidometric test in this study demonstrate 98.075% of *B. cereus* stains can product β-Lactamase. Results from the same previously study demonstrate high prevalence β-Lactamase in *B. cereus* strain. According to the same result study previously, 98 and 92% of *B. cereus* strains isolated from hospital surfaces and staff skin respectively, can produce β-Lactamase enzyme (Jalalpoor et al., 2009d, e, 2010).

Our finding in this study showed 57.1% of *B. cereus* strain isolated from high contact surface. According to the same result study previously, about bacterial epidemiology in hospitals, *Bacillus* spp. and *Staphylococcus* spp. were the most bacterial that isolated from hospital surfaces and staff skin (Jalalpoor et al., 2009e,d; Mansuri et al., 2007; Nasiry, 2000). Inappropriate use of disinfectant agents, due to increase resistance bacteria to susceptibility to this agent, and 14.3% of *Bacillus* spp. isolated from staff hands, with attention to role of these bacteria in different disease food poisoning and specially, Nosocomial infections it's necessary to go on to reduce prevalence, transmission and antibiotics resistance for this bacteria. We suggest that, improve quality of user disinfectant and hand washing agents in hospitals. Washing hands properly reduces the chance of getting infected or spreading infection. Result demonstrate high prevalence of resistance to β-Lactame antibiotics *B. cereus* strain, avoiding the use of antibiotics, in some situations, can also reduce the chances of infection by antibiotic resistant bacteria.

Penicillin is first selective antibiotic to cure of *Bacillus* infections diseases and according to result, it showed that 81% of *B. cereus* strains were resistance to Penicillin. Unnecessary use of β-Lactame antibiotic due to increase resistant in *B. cereus* strain, we suggest, physicians go on to with more carefully prescription antibiotics. High prevalence β-Lactamase in *B. cereus* strains, represent increase resistant into widespread spectrum into antibiotics. Increase contact of Bacteria, due to increase of transfer plasmids and creative resistant strains. We offer despite improve, act of infection control issue, go on to, reduce spread *B. cereus* in hospitals.

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