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Emergence of Antibiotic Resistance Nano Enzyme in *Staphylococcus* species Isolated from Clinical, Biotic and Abiotic Conditions

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ABSTRACT

Health care workers and hospital surfaces have important role in nosocomial infection, if resistance strain transfer to hospitalized patients, led to spread of antibiotic nosocomial infections. The research was laboratory and performed during 2005/2007 years in Azzahrahospital in Isfahan. According to statistical formula study on147 *Staphylococcus* species isolated from clinical samples, skin hands of health care workersandhospital surfaces. Bacterial identification, were performed with microbiological methods and bet lactamase product was performed with Acidometric method. According to Acidometric result 100% and 50% of *S. aureus* and *S. epidermidis* isolated from clinical samples and 75% and 66.6% of *S. aureus* and *S*. epidermidis isolated from skin hands of health care worker and 84.6%, 70.45% and 75% of S. *aureus, S. epidermidis and S. saprophyticus* isolated from hospital surfaces can produce beta lactamase Nano enzyme. Result demonstrate high prevalence of resistance antibiotics *Staphylococcusspp*. isolated from clinical, biotic and abiotic conditions in hospital. One of reason creative antibiotic resistant in bacteria is increase contact of sensitive bacteria with resistance strains.

Key words: *Staphylococcus aureus, S. epidermidis*, *S. saprophyticus,* Antibiotic Resistance, Beta lactamase Nano Enzyme.

INTRODUCTION

Antibiotic resistance is a consequence of evolution via natural selection or programmed evolution. 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 (Jalalpoor et al., 2007, Keith., 2005, Kim et al.,2000, Madani., 2009, Mielke., 2010). Several studies have demonstrated that patterns of antibiotic usage greatly. The several main mechanisms by which micro-organisms exhibit resistance to antimicrobials are: Drug inactivation or modification: e.g. enzymatic deactivation of Penicillin G in some penicillin resistant bacteria through the production of betalactamases(Jalalpoor et al 2007). Beta lactam antibiotics are typically used to treat a broad spectrum of Gram-positive and Gram-negativ bacteria. Beta-lactamases produced by Gramn egative organisms are usually secreted. Betal actamases are enzymes produced by some bacteria and are responsible for their resistance to beta-lactam antibiotics like penicillins, cephamycins, and carbapenems (ertapenem). (Cephalosporins are relatively resistant to betalactamase.) These antibiotics have a common element in their molecular structure: a four-atom ring known as a beta-lactam(George.,2005, Jalalpoor et al 2007, Mendelsonetal.,2005, Paterson etal.,2004).The beta lactamase enzyme breaks that ring open, deactivating the molecule's antibacterial properties. Penicillinase is a specific type of beta lactamase, showing specificity for penicillins, again by hydrolysing the beta-lactam ring. Molecular weights of the various penicillinases tend to cluster near 50kDN (George.,2005,

Jalalpoor et al 2007, Mendelson et al., 2005, Paterson et al.,2004). Penicillinase was the first betalactamase to be identified: it was first isolated byAbraham and Chain in 1940 from Gram-negative E. coli even before penicillin entered clinical use but penicillinase production quickly spread to bacteria that previously did not produce it or only produced it rarely. Penicillinase-resistant betalactams such as methicillin were developed, but there is now widespread resistance to even these (George., 2005, Mendelson et al., 2005, Paterson et al., 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. Staff hands and hospital surfaces have important role in NIs (Boyceet al., 2002; Ducel et al., 2002; Johnson 2006; Kampf et al.,2004; Stone et al.,2002). The health-care environment contains a diverse population of microorganisms. 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., via hand transferred .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 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 transferal of microorganisms from environmental surfaces to patients is largely via contact hands of staff with the surface(Boyceet al., 2002; Ducel et al., 2002; Johnson 2006;Jalalpoor et al., 2007; Kampf et al., 2004;Sehulster and Raymond, 2003;Stone et al., 2002). *S. aureus* is the most common grampositive bacterium causing nosocomial infections (Nis) (Mayon et al.1988; Steinbrecher et al.,2000). Its frequency among all pathogens in Nis variesbetween 11.1 and 17.2% (Ruden et al.,1995; Sartor et al.,1995; Wagner et al.,1997). Methicillin resistancein *S. aureus* (MRSA) is increasing worldwide (Schmitz et al.,1999)leading not only to NIs but recently also to communityacquiredinfection. Colonization of health care workers' hands with *S. aureus* hasbeen described to range between 10.5 and 78.3%. Upto 24,000,000 cells can be found per hand (Ayliffe et al.,1988). The colonizationrate with *S. aureus* was higher among doctors (36%) than among nurses (18%), as was the bacterial density of *S. aureus* on thehands (21 and 5%, respectively, with more than 1,000 CFU perhand) (Daschner et al.,1985). The carrier rate may be up to 28% if the healthcare worker contacts patients with an atopic dermatitis whichis colonized by *S. aureus* (Williams et al.1999). MRSA has been isolatedfrom the hands of up to 16.9% of health care workers. VRE canbe found on the hands of up to 41% of health care workers. Hand carriage of pathogens such as *S. aureus*, MRSA, or *S. epidermidishas* repeatedly been associated with different types of NI (Hilton et al.,2002).S. aureus can survive on hands for at least 150 min. On inanimate surfaces, S. aureus and MRSA may survive for 7 months, withwild strains surviving longer than laboratory strains. The long survivalon surfaces, together with the relatively short survival onhands, suggests that contaminated surfaces may well be the sourceof transient colonization despite negative hand cultures. Subject of this paper was survey prevalence of beta lactamase Nano enzyme in

Staphylococcus species isolated from clinical samples, skin hands of health care workers andhospital surfaces of Azzahra hospital in Iran.

MATERIALS AND METHODS

Sampling

A total of 147*Staphylococcus* spp., 14speciesfrom clinical samples, 105species from hospital surfaces and 28species from skin hands

of health care worker were isolated of Azzahrahospital during of 2005-2007 years ((Jalalpoor et al 2007, 2009a-e, Sehulster and Raymond, 2003, Washington et al., 2006). Clinical sample were randomly collected, hospital surfaces samples were collected from high and low hospital contact surfaces with swab (Effective sampling of surfaces requires moistened swabs) in Tryptone Soya Agar (Merck) and skin hands of health care worker samples, were randomly collected from staff hand in Blood Agar (Merck) via Fingerprint Technique (Jalalpour and Ebadi 2010c., Sehulster and Raymond, 2003).

Bacterial strains

Identification bacteria were performed with microbiological methods e.g Gram stains, andbiochemical tests with the BioMerieux database system and use of differential medium. Specimen grows on sheep blood and EMB agars incubated at 37°C under aerobic conditions (Jalalpoor et al., 2007,Washington et al., 2006).

Beta Lactamase Nano Enzyme Detection

Acidometric test is a Diagnostic test for the rapid detection of the beta-lactamase inbacteria. This test is based on hydrolysis of the betalactam ring, which results in the production of penicilloic acid. This process causes acidification of the bacterial suspension, and changes the colour of the acid basic indicator phenol red. The red color of this indicator is present negative test and The yellow color of this indicator is present positive test(Jalalpoor etal 2007) (Fig 1).

RESULTS

According to result of present study frequency of *S. aureus* and *S. epidermidis* in clinical samples were 12% and 2%, frequency of *S. aureus* and *S. epidermidisin* skin hands of health care worker were 5% and 30% and in hospital surfaces frequency of *S. aureus*, *S. epidermidis* and *S.*

Fig. 1. Beta Lactamase Nano Enzyme Production with Acidimetric Method

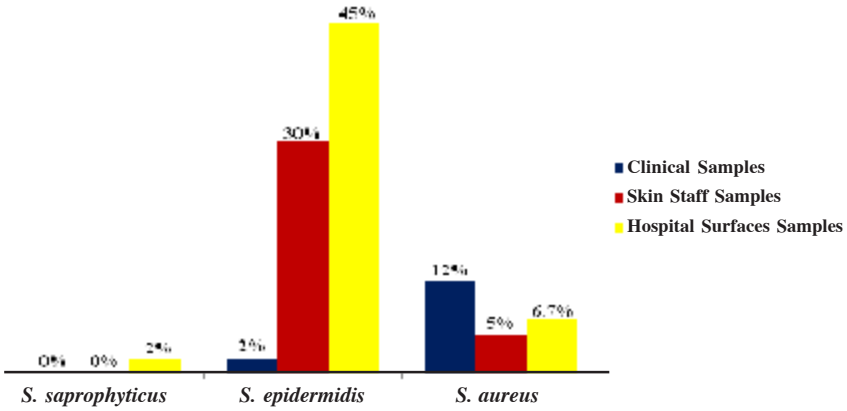

Fig. 2. Frequeence of *Staphylococcus* spp. in Samples

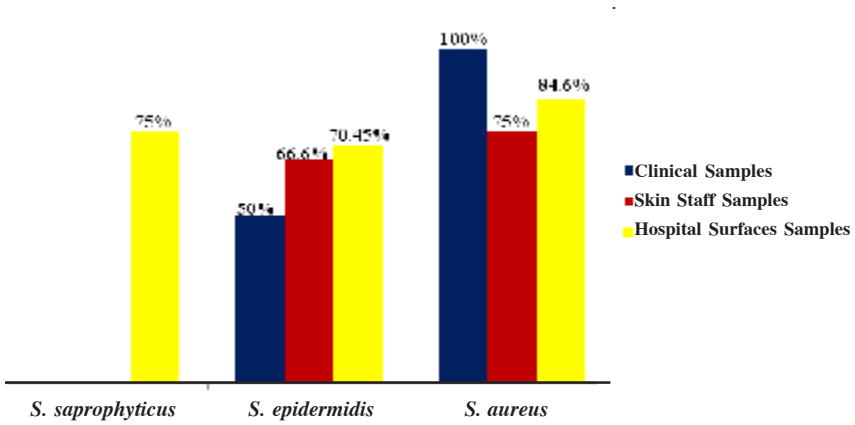

Fig. 3. Frequeence of Beta lactamase Nano Enzyme in *Staphylococcus* spp.

saprophyticus were 6.7%, 45% and 2% respectively (Fig 2). According to Acidometric result 100% and 50% of *S. aureus* and *S. epidermidis*isolated from clinical samples and 75% and 66.6% of*S. aureus* and *S. epidermidis*isolated from skin hands of health care worker and 84.6%, 70.45% and 75% of *S. aureus, S. epidermidis* and *S. saprophyticus* isolated from hospital surfaces can produce Beta Lactamase (Fig 3).

CONCLUSION

According result previous study in Iranian hospital, *Staphylococcus*sp. consist of isolated bacteria from hospital surfaces and 28 consist of isolated bacteria from hands of staff and according to Acidimetric test results respectively 73 species of *Staphylococcus* isolated from hospital surfaces and 19 species of *Staphylococcus* isolated from hands of staff was resistance to beta lactame antibiotics(Jalalpoor et al 2007). According previous study 83.33% of Staphylococcus spp. isolated from nosocomial infection in Iran was resistance to beta lactame antibiotics (Jalalpoor et al 2007). According result another study in Iran, 61.9% of bacteria isolated from biotic condition in hospital was resistance to beta lactame antibiotics, respectively was in *Staphylococcus* spp., *Bacillus* spp. and Enterobacteriaceae 71%, 64.72% and 50%, According another study in Iran 77.94% of Bacteria isolated from abiotic condition in hospital was resistance to beta lactame antibiotics, respectively was in *Staphylococcus*spp., Bacillus spp. and Enterobacteriaceae 82.7%, 68.4% and 80.35% (Jalalpoor etal 2007). Antibiotic resistance is an important tool for genetic engineering. By constructing a plasmid which contains an antibiotic resistance gene as well as the gene being engineered or expressed, a researcher can ensure that when bacteria replicate, only the copies which carry along the plasmid survive. This ensures that the gene being manipulated passes along when the bacteria replicates, the most commonly used antibiotics in genetic engineering are generally "older" antibiotics which have largely fallen out of use in clinical practice. These include: ampicillin, kanamycin, tetracycline and chloramphenicol (Boyce et al., 2002; Jalalpoor et al.,2007; Sehulsteret al.,2003). Industrially the use of antibiotic resistance is disfavored since maintaining bacterial cultures would require feeding them large quantities of antibiotics. Instead, the use of auxotrophic bacterial strains (and function-replacement plasmids) is preferred, 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 (Boyce et al.,2002; Jalalpoor et al.,2007; Sehulster et al.,2003). 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 (Madani etal.,2009, Mielke, 2010, Rosenthal et al., 2010a ,b, Victor et al., 2010).

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