

Prevalence of Beta Lactamase Nano Enzyme in Bacteria Isolated from Staff Hand (Isfahan-Iran)

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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. β -lactame antibiotics (Penicillin-Cephalosporin) have selective poisoning and effective to more Bacteria then are very important in cure of diseases. b-lactamase is virulence agent and causes resistance to these antibiotics. According rule of staff hand Bacteria in infection chain, transmission of β -lactamase producing Bacteria in patients, final due to β -lactame antibiotics resistance nosocomial infection in hospital. The subject of this study was survey prevalence of Beta lactamase nano enzyme in isolated *bacteria* from staff hand of AZZAHRA Hospital in Iran. The present study was performed at one tertiary care hospitals in Isfahan, Iran. During a 24 month period (2007 -2009) and 80 of *bacteria* isolated from staff hand were studied. Samples collected with finger print method. Standard microbiological methods were performed for detection of bacterial species and for determine β -lactamase production, use Acidimetric method. The collected data was analyzed thorough SPSS version 14 software and Chi-square used for determination of significance of association. The $p < 0.05$ was considered significant. According to result, *Staphylococcus* spp. 28 (35%), *Bacillus* spp. 48 (60%) *Enterobacteriaceae* 4 (5%) consist of isolated bacteria. According result of acidometric test from 80 isolated staff hands bacteria 61.9% of strains produce β -lactamase, respectively was in *Staphylococcus* spp., *Bacillus* spp. and *Enterobacteriaceae* 71%, 64.72% and 50%. Establish systems for monitoring antimicrobial resistance in hospitals and the community and link these findings to resistance and disease surveillance data is fundamental to developing treatment guidelines accurately and to assessing the effectiveness of interventions appropriately.

Key Words: β -lactame Antibiotics, b-lactamase, Staff Hand, Nosocomial Infection.

Nosocomial infections 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 hand and hospital surfaces have important role in NIs, 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 condition¹⁻⁵. 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 transferral .

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The staff hand 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, based on the principles of the chain of infection, is also important in evaluating the contribution of the environment to health-care-associated disease¹⁻⁷. 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 transfer 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 transferral of microorganisms from environmental surfaces to patients is largely via hand contact with the surface^{11,12}. The most important and frequent mode of transmission of nosocomial infections, is divided into two subgroups: direct-contact transmission and indirect-contact transmission^{11,12}. 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^{11,12}.

Total counts of bacteria on the hands of medical staff have ranged from 3.9×10^4 to 4.6×10^6 . Their number increases with the duration of clinical activities, on average by 16 cells per min. Between 4 and 16% of the hand surface is exposed by a single direct contact, and after 12 direct contacts, up to 40% of the hand surface may have been touched. The transmissibility of transient bacteria depends on the species, the number of bacteria on the hand, their survival on skin, and the dermal water content. Duration of persistence

on inanimate surfaces in *E. coli* is 2 h–16 months, in *Klebsiella* spp. is 2 h–30 months, in *S. aureus* is 4 wk–7 months, in VRE is 5 days–4 months, and in Spore-Forming Bacteria in vegetative cells are at least 24 h and spores survive for up to 5 months¹³.

Antibiotic resistance can also be introduced artificially into a micro-organism through transformation protocols. This can be a useful way of implanting artificial genes into the microorganism, 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 generation¹⁴⁻¹⁷. 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 β -lactamases¹⁴⁻¹⁷. Beta-lactam antibiotics are 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 (EC 3.5.2.6) 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 beta-lactamase.) These antibiotics have a common element in their molecular structure: a four-atom ring known as a beta-lactam¹⁷⁻²¹.

The lactamase enzyme breaks that ring open, deactivating the molecule's antibacterial properties. Penicillinase is a specific type of β -lactamase, showing specificity for penicillins, again by hydrolysing the beta-lactam ring. Molecular weights of the various penicillinases tend to cluster near 50kDALTON¹⁸⁻²². Penicillinase was the first β -lactamase to be identified: it was first isolated by Abraham 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 beta-lactams such as methicillin were developed, but there is now widespread resistance to even these¹⁸⁻²².

The subject of this study was survey prevalence of Beta lactamase nano enzyme in isolated *bacteria* from staff hand of AZZAHRA Hospital in Iran.

Methods

Sampling

A total 80 bacteria from staff hand were isolated in Azzahra-hospital in Esfahan during of 2007-2009 years.

Staff hand samples, were randomly collected from staff hand and cultured in Blood Agar and EMB Agar (Merck) via Fingerprint Technique^{12,23,24}.

Bacterial strains

Identification Bacteria, were performed with microbiological methods e.g Gram stains, biochemical tests with the BioMerieux database system and use of differential medium. All of Specimen in firs step grows on sheep blood and EMB agars incubated at 37°C under aerobic conditions^{12,26}.

Detection Beta Lactamase

Acidometric test is a Diagnostic test for the rapid detection of the β-lactamase in bacteria^{12,24}.

This test is based on hydrolysis of the β-lactam ring , which results in the production of penicilloic acid. This process causes acidification of the bacterial suspension, and changes the colour of the acidobasic indicator phenol red. The result of the reaction is very fast. The red colour of this indicator is present negative test and The yellow color of this indicator is present positive test^{12,25} (Fig. 1).



Fig. 1. Acidimetric Method

Table 1. Frequency of bacteria in staff hand

Bacteria	<i>Enterobacteriaceae</i>		<i>Bacillus</i> spp.		<i>Staphylococcus</i> spp.							
Number / Percent	%	N	%	N	%	N						
	5%	4	60%	48	35%	28						
Species	<i>K.pneomonieae</i>		<i>E.coli</i>		<i>B.cereus</i>		<i>Bacillus</i> sp		<i>S.aureus</i>		<i>S.epidermidis</i>	
Number/ Percent	%	N	%	N	%	N	%	N	%	N	%	N
	5/2%	2	5/2%	2	25/16%	13	75/43%	35	5%	4	30%	24

Statistical analyze

All the statistical analyses carried out using SPSS version 14. And Chi-square used for determination of significance of association. The pd” 0.05 was considered significant.

RESULTS

According to result, *Staphylococcus* spp.

28 (35%), *Bacillus* spp. 48 (60%) *Enterobacteriaceae* 4 (5%) consist of isolated bacteria (Table 1). According result of acidimetric test from 80 isolated staff hands bacteria 61.9% of strains produce β-lactamase, respectively was in *Staphylococcus* spp., *Bacillus* spp. and *Enterobacteriaceae* 71%, 64.72% and 50% (Fig. 2).

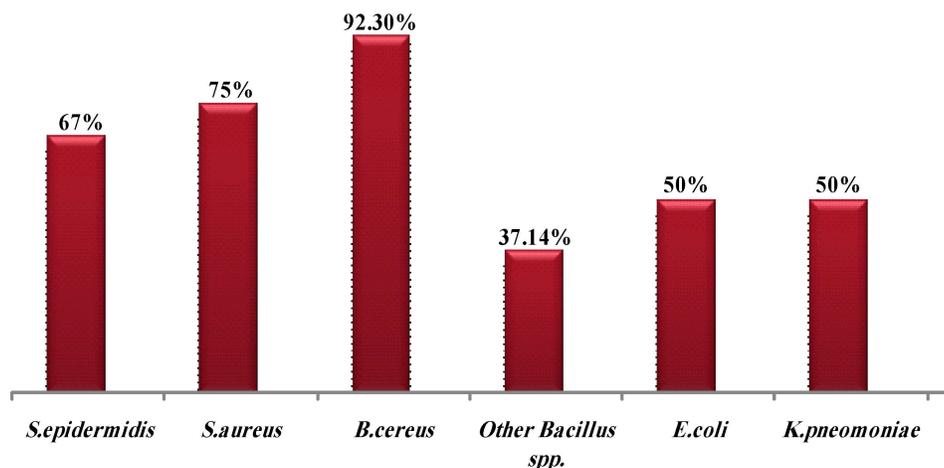


Fig. 2. Frequency of Beta Lactamase in bacteria isolated from staff hand

CONCLUSIONS

Result of this study show high frequency of antibiotic resistant strains on staff hand. Comparing the results obtained in this research with the results obtained in previously published articles that are similar to this research.

Result of this study demonstrate *Staphylococcus* spp. 28 (35%), *Bacillus* spp. 48 (60%) *Enterobacteriaceae* 4 (5%) consist of isolated bacteria.

According result the same previously study about bacterial epidemiology in hospitals in Iran *Bacillus* sp. and *Staphylococcus* sp. were the most bacterial that isolated from staff hand and hospital environment^{24,28,29}.

According result the same previously study about bacterial epidemiology in staff hand in other country demonstrate colonization of health care workers' hands with *S. aureus* has been described to range between 10.5 and 78.3%. Up to 24,000,000 cells can be found per hand, the colonization rate with *S. aureus* was higher among doctors (36%) than among nurses (18%), as was the bacterial density of *S. aureus* on the hands (21 and 5%, respectively, with more than 1,000 CFU per hand)¹³. The carrier rate may be up to 28% if the health care worker contacts patients with an atopic dermatitis which is colonized by *S. aureus*, *S. aureus* can survive on hands for at least 150 min; VRE survives on hands or gloves for up to 60 min¹³.

Colonization rates of gram negative bacteria on the hands of health care workers have been described as ranging from 21 to 86.1%, with the highest rate being found in ICUs¹³. The number of gram negative bacteria per hand may be as large as 13,000,000 cells, the colonization may be long-lasting, even in nursing homes, a rate of 76% has been described for nurses hands. Different species of gram-negative bacteria exhibit different colonization rates, for instance, the colonization rate is 3 to 15% for *Acinetobacter baumannii*, 1.3 to 25% for *Pseudomonas* spp., and 15.4 to 24% for *Serratia marcescens*, *Klebsiella* spp. were found on the hands of 17% of the ICU staff sampled, with up to 10,000 bacteria per hand, most gram-negative bacteria survive on the hands for 1 h or more¹³.

During a third outbreak, caused by *Bacillus cereus* in a neonatal ICU, 11 (37%) of 30 fingerprints from health care workers were positive for *Bacillus* spp.¹³.

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^{15,12}. Modern infection control is grounded in the work of Ignaz Semmelweis, who in the 1840s demonstrated the importance of hand hygiene for controlling transmission of infection in hospitals, the importance of hands in the transmission of hospital infections has been well demonstrated and can be

minimized with appropriate hand hygiene^{15,12}. 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 inactivation³⁰⁻³³. 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^{15,12}.

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