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Staphylococcus epidermidis is more severe than the Balinese morphology and endometriosis

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Abstract

Background and aim: *The ability to form biofilm is increasingly recognized as an important pathogenic factor in Staphylococcus epidermidis. However, the role of biofilm determinants in biofilm formation is very contradictory and diverse. The present study was conducted with the aim of investigating the polymorphism and pathogenic genes of Staphylococcus epidermidis strains isolated from clinical samples.*

Methods: *From 520 clinical samples including: urine, blood, feces and wounds of patients admitted to Tehran hospitals, they were cultured and then analyzed using bacteriological and biochemical tests to identify and isolate Staphylococcus epidermidis. The abundance of icaA, icaB, icaC, icaD genes was investigated by multiple PCR technique. Then BOX-PCR was performed to identify dominant types using specific oligonucleotide sequences.*

Findings: *60 isolates of Staphylococcus epidermidis were isolated from clinical samples after culture and diagnostic tests. Most isolates were obtained from urine samples with a frequency of 45%. icaD gene was the most abundant with 65%. The frequency of other studied genes was icaC (50%), icaB (13.33%), icaA (11.66%), respectively. The simultaneous presence of ica D and ica C genes (40%) was more than other genes. The simultaneous presence of icaA, icaB, icaC and icaD genes was not detected in any of the investigated isolates. ica A and ica D were observed simultaneously in 10% of the investigated isolates. In examining the genomic polymorphism of Staphylococcus epidermidis strains, 10 clusters were identified by BOX-PCR method. Cluster 8 with 17 isolates contained the most abundant isolates. After that, cluster 4 was the most common with 10 isolates. Cluster 9 and 10 each had 9 isolates. Cluster 1 and 3 each with one isolate had the lowest frequency.*

Conclusion: Based on the findings, Staphylococcus epidermidis strains are genetically diverse and this shows the polyclonal prevalence of strains in clinical samples. Also, BOX-PCR is a suitable method for molecular typing of Staphylococcus epidermidis strains and determining the foci of infection, which can be used for epidemiological studies and determining infection control strategies.

Keywords: *Staphylococcus epidermidis, icaA, icaB, icaC and icaD, BOX-PCR*

Introduction

Staphylococci are among the first pathogenic bacteria whose biochemical characteristics were known in the early 1880s. In the 1986 edition of Burji's book Systematic Bacteriology, the genus Staphylococcus was placed in the family Micrococcaceae with the three genera Micrococcus, Planococcus and Stomatococcus, but various genetic and chemotaxonomic studies later showed that these four genera should not be placed in a single family. 1), therefore, in the new edition of this book, the genus Staphylococcus is included in the family Staphylococaceae along with the genus Gemla (2).

Staphylococci are widespread in nature and are often present as normal microflora of the skin and mucous membranes in the nose and upper part of the respiratory tract in humans and animals. (3).

The genus Staphylococcus has more than 35 species, of which four species, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Staphylococcus saprophyticus*, and *Staphylococcus hemolyticus* are of special clinical importance (3). Staphylococci are Gram-positive cocci that have a diameter of about 0.5 to 1.5 microns and are observed in single, double, quadruple and chain forms in liquid culture medium. These bacteria are usually put together in the form of irregular communities and in the form of grape clusters, which are created in several levels due to the way staphylococcus cells divide (4, 5)

Clinically, it is important to distinguish staphylococci from micrococci. Micrococci are non-motile Gram-positive cocci, obligate aerobic, catalase-producing and coagulase-negative, which usually have yellow, orange or red colored colonies in culture media. These bacteria are not pathogenic, but it is necessary to distinguish them from coagulase-negative staphylococci (CoNS) (4, 9).

Among the differences between Staphylococcus and Micrococcus is the chemical composition of the cell wall. Staphylococci have an intermediate link containing pentaglycine in the peptide bridges of peptidoglycan, which is not present in micrococci. The percentage of G+C in micrococci is about 37% to 38%, which is almost twice that in staphylococci (4, 5, 8).

Coagulase-negative staphylococci (CoNS) include various species, most of which are the normal flora of the skin and mucous membranes of humans and other mammals. These bacteria were first described in 1884 by Rodenbach and named as a non-pathogenic species called Staphylococcus albus. For a long time, these organisms were considered only as contaminants of culture media, so that even their isolation from samples that are sterile under normal conditions was also considered as contamination (10-12).

Methods:

Types of study

The current study was descriptive-sectional type (Cross-Sectional) which was conducted in the form of basic science studies (Experimental).

Statistical population

In this research, 520 different clinical samples including urine, blood, feces and wound swabs were collected from patients hospitalized in Tehran hospitals under completely sterile conditions.

Cultivation and purification of bacteria

The samples were cultured on blood agar medium and incubated for 24 hours at 37 degrees Celsius, and diagnostic tests were performed on the obtained colonies to identify Staphylococcus epidermidis. Then, a warm stained slide was prepared from the colonies and observed under a microscope. The following tests were performed for gram-positive cocci with a regular cluster arrangement.

Gram stain

In order to identify staphylococcal strains, after purification and preparation of fresh cultures from hospital samples and healthy individuals, Gram stain slides were prepared from the colony and examined under a microscope and stained in this way. The coloring steps include:

1. First, one or more pure colonies were placed on a sterile slide on which we placed a drop of sterile distilled water and dissolved in distilled water.

2. After drying and fixation, the samples were stained with crystal violet for 45 seconds to one minute.

3. After this period, they are washed slowly, then Lugol's solution was poured on the slide for 60 seconds.

4. After that, the slide was washed with water in an alcohol-acetone solution and then stained with safranin for 30 seconds to one minute.

5. At the end, wash the slide slowly with water and wait for a while until the slide is completely dry.

6. After that, the slide was examined under a microscope with a 100 lens.

catalase test

Catalase test is used to differentiate Staphylococcus and Micrococcus from Streptococcus.

In this method, a drop of 3% hydrogen peroxide solution (H2O2) was placed on a glass slide and a colony of bacteria was dissolved inside it. The formation of air bubbles indicates a positive result of the reaction, which is due to the production of catalase enzyme by the bacteria. This test is positive for Staphylococcus and Micrococcus.

Bacitracin resistance test

To differentiate staphylococci from micrococci, bacitracin 0.04 µg diagnostic disc was used by disc diffusion method. Staphylococcus bacteria are resistant to this antibiotic, while Micrococcus is sensitive to bacitracin.

Results:

Sampling and isolation of coagulase-negative staphylococci

In this study, 60 samples of Staphylococcus epidermidis were collected from urine, blood, feces and wound swabs from patients hospitalized in Tehran hospitals under completely sterile conditions. The most strains were isolated from the urine sample with a frequency of 45%

The results of Staphylococcus epidermidis identification tests

Bacteria isolated from different clinical samples were identified and purified using gram staining and biochemical tests (catalase, coagulase, resistance to bacitracin, resistance to polymyxin B, sensitivity to novobiocin, fermentation of mannitol, PYR, ODC, fermentation of sugars). Colonies of this bacterium on blood agar medium were opaque, white, round and large without hemolysis (Figure 1). Also, these bacteria were catalase positive (Figure 2), coagulase negative, urease positive, ornithine decarboxylase positive, PYR positive. (Figure 2) and is sensitive to nobiocin The results of the sugar fermentation test for the sugars maltose, sucrose, trehalose and mannitol were as follows: maltose: positive, sucrose: positive, trehalose: negative and mannitol: negative.

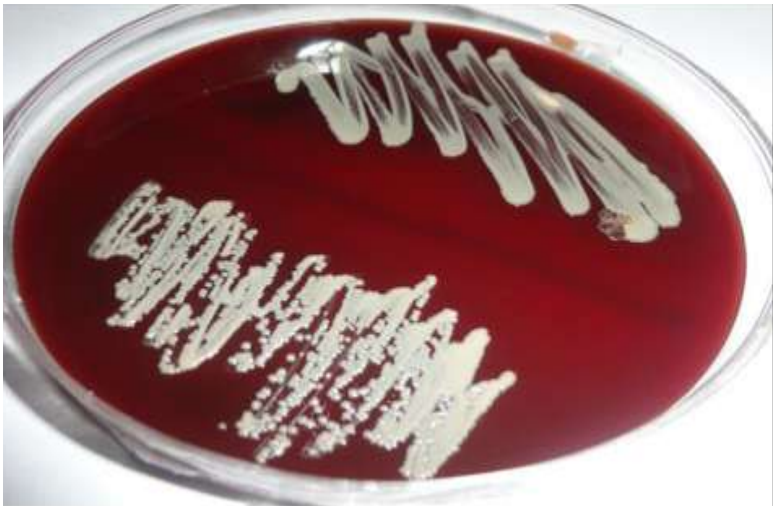

Figure 1 Staphylococcus epidermidis bacteria culture on blood agar

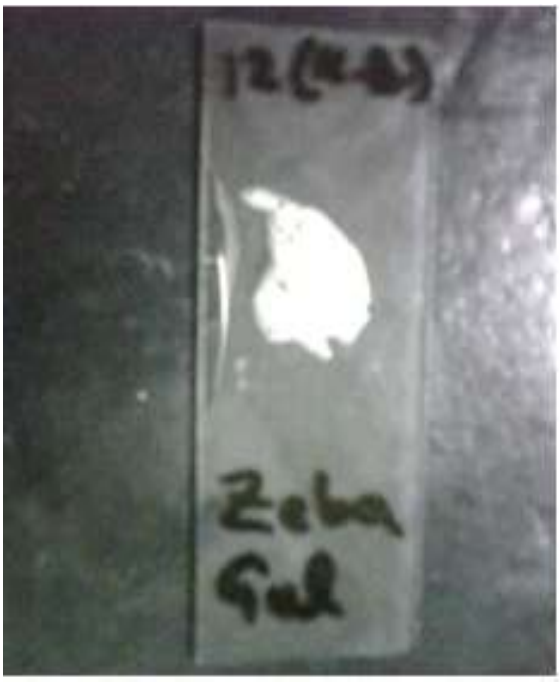

Figure 2 Staphylococcus epidermidis catalase test

Discussion

Staphylococcus epidermidis is the most abundant coagulase-negative staphylococcus (CoNS) isolated from humans. This microorganism is part of the natural flora of the human skin and mucous membrane, which has the ability to cause disease in people with immune deficiency or in people who have damage caused by foreign bodies. In recent years, due to the increase in medical interventions, such as the use of vascular catheters and prosthetic device implants, the prevalence of Staphylococcus epidermidis infections has increased significantly. As a result, this organism is increasingly isolated and identified as a pathogen causing nosocomial sepsis. So that approximately 30% of hospital infections are blood stream (13). This organism is also associated with various clinical manifestations such as late-onset sepsis in premature infants, central nervous system shunt infection, endocarditis, urinary tract infection, surgical site infection, and endophthalmitis. Although Staphylococcus epidermidis is an opportunistic organism, this microorganism has several pathogenic factors such as hemolysin, lipase, protease, lecithinase, DNase and toxin (14). One of the special features of Staphylococcus epidermidis is the ability to adhere to polymer surfaces and subsequently form a biofilm. Strong attachment of bacterial biofilm to polymer surfaces is the first stage of intravascular catheter-related bacteremia and other device-related infections, leading to sepsis (15). A biofilm consists of layers embedded in a matrix of extracellular polysaccharide (slime) that facilitates bacterial adhesion to surfaces, protects the host's immune response, and acts as an efficient barrier against antibiotics. Therefore, eradicating bacteria in biofilms is difficult, as resistance to antibiotics eventually leads to removal of infected devices. Biofilm formation is regulated by the expression of intercellular adhesion polysaccharide

(PIA). PIA is composed of β-1,6 N-acetylglucosamine and is responsible for cell-cell adhesion and is essential for biofilm formation in S. epidermidis strains (16). PIA is encoded by the chromosomal intercellular adhesion (ica) locus, which consists of the structural genes icaADBC and the regulator icaR. Among them, icaA and icaD genes, having enzyme activity (N-acetylglucosaminyltransferase), play a central role in biofilm production. icaA alone has little enzymatic activity, but simultaneous expression with icaD activates the activity of N-acetylglucosaminyltransferase and produces oligomers with a length of 20 residues (17). In other words, the product of the icaA gene is a membrane protein with homology to N-acetyl-glucosaminyltransferases, which requires the product of the icaD gene for optimal activity. N-acetylglucosamine oligomers produced by icaAD reach a maximum length of 20 amino acids, and longer oligomeric chains are synthesized only when icaAD is expressed with icaC, which encodes a putative membrane protein. icaC is also likely to be involved in transporting the growing polysaccharide to the cell surface. Then the surface-bound protein IcaB is responsible for the deacetylation of the poly-N-acetylglucosamine molecule. . Non-acetylated polyglucosamine in the isogenic icaB mutant strain cannot adhere to the bacterial cell surface or induce biofilm formation (18-20).

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