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Isolates of Staphylococcus Aureus Produce Biofilms

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Abstract

The capacity of Staphylococcus aureus to shape biofilm is viewed as a significant destructiveness factor impacting its endurance and steadiness in both the climate and the host. Biofilm development in S. aureus is most often connected with the development of polysaccharide intercellular bond by ica operon-encoded compounds. The purpose of this study was to determine whether or not S. aureus isolates from a dental clinic in Konya, Turkey, produced biofilms in vitro and contained the icaA and icaD genes. Over the course of six months, samples were taken from the surfaces of inanimate objects. S. aureus isolates were tested for biofilm production using crystal violet (CV) and Congo Red Agar (CRA) staining assays, and the presence of the icaA and icaD genes was determined using polymerase chain reaction. 13.2% of environmental samples contained S. aureus contamination. The icaA and icaD genes were found to be present in all isolates. Phenotypic assessments uncovered that CV staining measure is a more solid option in contrast to CRA examine to decide biofilm development capacity. The results of CV staining and the detection assays for ica genes were found to be highly compatible. In order to find out if S. aureus is forming biofilms, phenotypic and genotypic tests should be performed simultaneously. Our discoveries demonstrate that dental facility conditions ought to be considered as possible repositories for biofilm-delivering S. aureus and hence cross-defilement.

Keywords: Staphyloccocus aureus; Dental center surfaces; Genes for biofilms; Konya

Introduction

Microorganisms with the potential to cause disease could spread from the wounds or mouths of patients to the hands of dental professionals, eventually contaminating every surface in the dental office [1]. Aerosols have been reported to contribute to the environmental contamination of dental settings in addition to direct skin contact. When combined with a water spray, the propelling force of a high-speed dental drill and the cavitation effect of an ultrasonic scaler can produce numerous aerosols in which microorganisms can be suspended. The information got from a multi-seat dental center showed that dental vapor sprayers have the ability to spread quickly, even into regions where there is no dental movement.

The majority of bacteria have a propensity to stick to any surface that is available and readily form biofilms, which could be problematic in dental settings [2]. A biofilm is simply and broadly defined as a surface-attached community of microorganisms that maintain a protective level of homeostasis and stability in a changing environment. Compared to their planktonic counterparts, it has been demonstrated that bacterial cells in biofilms are more resistant to environmental stresses and antimicrobials. In a biofilm, the close proximity of bacterial cells may also facilitate communication between cells, promote horizontal gene transfer, and enable cooperative metabolic functions [3]. Biofilms are typically associated with surfaces that are wet or damp, like the tubing on medical equipment and indwelling medical devices. However, some bacterial species, such as Staphylococcus aureus, can form biofilm on dry clinical surfaces and remain viable for extended periods in a desiccated state.

S. aureus is a human pathogen that thrives in the skin and mucous membrane microbiota and is capable of causing a variety of healthcare-associated infections (HCAIs) in people of all ages. S. aureus is increasingly being recognized as a cause of severe invasive diseases like osteomyelitis, septic arthritis, and pneumonia with empyema in addition to the infections of the skin and soft tissues. In addition, over the past few decades, multi-drug-resistant S. aureus has increased in both healthcare and community settings.

Staphylococci, in particular S. aureus, have been shown to possess the ability to form biofilms in numerous instances [4]. In clinical staphylococci, the presence of biofilm development is viewed as significant for endurance and harmfulness. Many types of S. aureus contamination are related with the development of a bacterial biofilm on either local tissues or embedded biomaterials. Creation of a polysaccharide grip, named polysaccharide intercellular bond (PIA), by ica operon-encoded catalysts is right now the best-perceived component of staphylococcal biofilm improvement.

Numerous studies have examined S. aureus contamination of dental clinic surfaces. Until now, be that as it may, there is no distributed work on the portrayal of biofilm arrangement by S. aureus strains disconnected from dental center surfaces. As a result, phenotypic and genotypic assays were used in this study to evaluate the biofilm properties of S. aureus isolates taken from abiotic surfaces in a dental clinic.

Materials and Methods

Environmental sampling

A total of samples were taken from the surfaces of inanimate objects in the clinical areas of a dental clinic at Necmettin Erbakan University, Konya, Turkey, Faculty of Dentistry. The likelihood of bacterial contamination and accumulation was taken into consideration when selecting surfaces. Examining configuration included surfaces of alginate holder, amalgamator, counter, dental-seat press button, glove compartment, light handle, pull hose, towel allocator and X-beam tube.

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Sampling was carried out at regular intervals between clinical activities and end-of-day cleaning and disinfection procedures [5]. Sterile cellulose sponges (4 x 8 cm) pre-moistened with neutralizing buffer were used to collect samples on each sampling day from three representative locations for each surface. As a result, the number of samples taken from each surface type was equal. The sponges were immediately transported to the laboratory in insulated cooler boxes after being returned to their individual plastic bags following the sampling.

Isolation and identification of S. aureus

Each plastic bag containing the sponges was infused with one hundred milliliters of Trypticase Soy Broth. Initial suspensions were streaked onto Mannitol Salt Agar plates after the contents of the bags, which represented the initial suspensions, were mixed and incubated. To check for purity, presumptive S. aureus colonies with yellow zones were restreaked onto Trypticase Soy Agar plates after 48 hours of incubation at 35 °C. The Staph-ID system was then used to biochemically identify the pure cultures that were grown on TSA plates.

Culture conditions

S. aureus strain ATCC and S. epidermis strain, provided by Microbiologics Inc, were remembered for the concentrate as biofilm-positive and biofilm-pessimistic controls, individually [6]. At a temperature of 18 °C, stock cultures of reference strains and isolates were kept in TSB with 20% glycerol added. Working cultures were grown at 4 °C on TSA slants.

The isolates were cultured on congo Red Agar (CRA) plates containing 36 g/L saccharose (Merck) and 0.8 g/L Congo Red dye for qualitative slime formation detection. Separates were vaccinated on CRA plates and brooded vigorously. Isolates that produce slime form black colonies, whereas those that do not produce slime form red colonies.

Crystal violet staining assay isolate overnight cultures were diluted 100 times in TSB with 2 percent (w/v) glucose [7]. 200 milliliters of the diluted cultures were poured into the wells of a sterile polystyrene microplate with wells (Anicrin, Venezia, Italy). Nonadherent cells were removed from the wells by washing them three times with Phosphate-Buffered Saline (PBS) after incubation. After that, the washed wells were air-dried, and a crystal violet (CV) staining assay was used to measure the levels of biofilm biomass on the well surfaces. The biofilm's bacterial cells were fixed with ethanol and stained for five minutes with 1% crystal violet (Merck). Wells were air-dried after being stained three times with sterile distilled water [8]. At long last, bound CV was delivered by adding 95% ethanol, and the degree of CV in not entirely settled by estimating the optical thickness (OD) at a frequency of 595 nm utilizing a spectrophotometer. All isolates underwent the quantitative biofilm assay in triplicate. The biofilm-negative control strain's mean plus three standard deviations was used as the cut-off OD value (ODc). Biofilm-producing isolates were those with a mean OD value greater than ODc.

Results and Discussions

In recent years, it has become increasingly accepted that contaminated surfaces are involved in the acquisition of HCAIs and play a significant role in the transmission of clinically relevant pathogens [9]. One of the most prevalent pathogens that causes HCAIs is S. aureus, and its genes include those for biofilm formation. In this study, biofilm-producing S. aureus strains were isolated from inanimate surfaces in a university dental clinic over the course of six months. S. aureus contamination was found in of the 243 environmental samples. Samples taken from the surfaces of dental chair push buttons had the highest positive contamination rate, followed by counters.

Extracellular polysaccharides—commonly referred to as "slime"—that appear to play an important role in bacterial adhesion are produced during the accumulation phase of biofilm formation. The CRA assay, a straightforward qualitative phenotypic method, was used to identify slime production in this study. Among detaches (one for every positive example), segregates created trademark dark settlements were listed as slime producers on CRA. The CV staining assay was used to quantify the ability of isolates to form biofilms. CV is an essential color, which ties to adversely charged surface particles and polysaccharides in both the extracellular framework and cytoplasm. The CV staining assay revealed that S. aureus isolates can form biofilms. The quantitative biofilm assay also categorized all slime-positive isolates as biofilm-positive, and red colonies were observed on CRA for biofilm-positive isolates [10]. Our outcomes were as per those of Arslan and Özkardeş who revealed that the CRA examine yielded lower level of positive outcomes in clinical staphylococci disconnects contrasted with the staining measure.

The level of arrangement (the proportion of the quantity of disengages that yielded comparative outcomes with the two examines to the all out number of secludes) between the aftereffects of CRA and quantitative biofilm not entirely set in stone as 56%. As per our outcomes, CRA was not suggested for the discovery of biofilm development in S. aureus strains because of an unfortunate connection between's state morphology and biofilm ph enotype in the quantitative biofilm examine. The different glucose concentrations in the test media can account for the disparity in results between the two assays. The percentage of biofilm-producing S. aureus was reported to have increased when percent glucose was added to the TSB. Consequences of a past report showed that higher centralizations of glucose emphatically upgraded the outflow of ooze development of S. epidermidis stresses on the CRA definitions with various glucose contents.

S. aureus' ability to form biofilm is controlled by the icaA and icaD genes, which mediate the synthesis of PIA, which is made up of linear -1,6-linked glucosaminylglycans. The enzyme N-acetyl-glucosaminyltransferase was shown in vitro to produce PIA from UDP-N-acetyl-glucosamine [11]. The icaA quality item is a transmembrane protein with homology to N-acetyl-glucosaminyltransferases, requiring the icaD quality item for ideal movement. In conventional agarose gel electrophoresis, a false-negative result may result from weak amplification of the target DNA. To avoid false-negative results while detecting the ica gene-specific PCR amplicons, we used a sensitive, high-resolution capillary electrophoresis device. Our outcomes demonstrated that every one of the 32 S. aureus disconnects held onto the icaA and icaD qualities, yielding the normal 1315 bp and 381 bp quality explicit enhancement items, separately. S. aureus strains isolated from clinical and environmental samples have been found to have a high prevalence of ica genes, which is consistent with our findings. In a new report, all of the 300 clinical disengages of S. aureus were accounted for to have the ica locus, as well as the icaA and icaD qualities. All of the clinical S. aureus isolates tested positive for both genes in a different study that was carried out in Turkey. The biofilm-forming capabilities of the isolates were used to characterize them. However, our findings contrast with the data that were presented, which only detected the icaA and icaD genes in isolates of S. aureus [12]. This disparity can be credited to the preliminaries utilized in their review, which depended on the grouping of the icaADBC got from S. epidermidis, as opposed to from S. aureus.

Conclusion

The genotypes and phenotypes of the isolates as determined by PCR and CV staining assays were found to be in good agreement (91 percent) in this study. The CV staining assays' broad applicability, dependability, and high reproducibility were previously demonstrated for bacterial biofilms. According to our findings, only three out of 32 genes-positive isolates lacked the capacity to form biofilm. In accordance, it has been demonstrated in vitro that S. aureus strains with the ica locus fail to form biofilm. These findings suggest that the expression of ica genes is strongly influenced by environmental factors like glucose, temperature, osmolarity, and growth in anaerobic conditions, and that biofilm production is regulated by the interaction of various regulatory mechanisms. The ica operon's transcriptional regulation is complex because of the interdependent and independent work of numerous activators and repressors. Differential transcriptional guideline of the locus or potentially putative ica-free biofilm components can impact biofilm creation aggregate. Point mutations in the ica locus and insertional inactivation have also been suggested as potential causes of S. aureus biofilm-negative variants. As a result, phenotypic and genotypic assays should be used together to identify biofilm-producing S. aureus isolates with greater certainty.

Acknowledgement

None

Conflict of Interest

None

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