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REVIEWER'S REPORT

Manuscript No.: IJAR-53009 Date: 25/07/2025

Title: Biofilm of staphylococcus aureus on different dentures materials

Recommendation:
Accept as it is ……………………………….
Accept after minor revision……Yes………..
Accept after major revision ……………..
Do not accept (*Reasons below*) ………

Rating	Excel.	Good	Fair	Poor
Originality	•			
Techn. Quality		•		
Clarity		•		
Significance	•			

Reviewer Name: Dr. Sireesha Kuruganti **Date:** 25/07/2025

Detailed Reviewer's Report

This review focuses on the formation of Staphylococcus aureus biofilms on various denture materials, discussing the implications for oral health, particularly denture stomatitis, and exploring factors influencing biofilm development and potential strategies for inhibition and eradication.

Here's a detailed, in-depth review of the manuscript:

Abstract Analysis

The abstract effectively introduces Staphylococcus aureus as an opportunistic pathogen proficient in biofilm formation on dental prostheses, highlighting the associated risks of antimicrobial resistance, systemic infections, and denture stomatitis. It notes the increased prevalence of denture stomatitis among denture users and describes it as an inflammatory reaction on the oral mucosa. The abstract then points to medical extracts with antibacterial action as a safer treatment option. It emphasizes the importance of reducing infection risks in immunocompromised cancer patients with maxillary abnormalities, and the need to understand how saliva affects microbial adherence to obturator materials, as well as developing materials with longer lifespans and reduced microbial attachment. The review's aim is clearly stated: to highlight various aspects of S. aureus biofilm development, its architecture, components, clinical consequences, involvement in pathogenesis and drug resistance, and to discuss strategies for its inhibition and eradication, including methodologies for qualitative and quantitative investigation.

Introduction: Staphylococcus aureus (Lines 28-57)

This section provides a comprehensive overview of Staphylococcus aureus. It is described as a Gram-positive opportunistic pathogen primarily colonizing the skin and mucous membranes, often asymptomatically in healthy individuals. It's identified as a leading cause of skin and soft tissue infections, especially in colonized individuals, and a major bacterial cause of death globally. Superficial skin infections can be gateways to fatal systemic infections, and S. aureus is a prevalent harmful

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bacterium in hospitalized individuals, contributing to high mortality and recurrence rates of invasive infections. Its capacity to develop and transmit multiple antibiotic resistance (AMR) is also noted.
The manuscript highlights that denture users have a higher likelihood of oral S. aureus colonization compared to non-wearers, and dental prostheses are a surface S. aureus can attach to. Dentures, being non-shedding oral surfaces, are particularly susceptible to staphylococcal biofilm colonization. S. aureus can cause various buccal region infections, including angular cheilitis, periodontitis, mucositis, and dental implant-linked infections. The text also mentions that infectious agents entering the respiratory system can lead to pneumonia. Historically, oral S. aureus colonization was thought to have no impact on oral health, but recent evidence suggests it's more frequent in the oral cavity than previously thought, potentially leading to systemic and oral illnesses. Within a biofilm, S. aureus produces an extracellular polymeric substance (EPS) that helps resist germs and lessens the effect of antibacterials. The dynamic evolution of S. aureus through mobile genetic components is emphasized, underscoring the need for uniform metadata in genomic databases for enhanced surveillance and a "One Health" approach to track its evolution, especially regarding the co-dissemination of resistance and biofilm genes across ecological niches.
Biofilm Formation in Staphylococcus aureus (Lines 64-77)
This section details the initial steps of S. aureus biofilm formation. It begins with the attachment of free-floating planktonic cells to a suitable surface, initiating colonization. Adherence is governed by physicochemical interactions, particularly hydrophobic and hydrophilic forces between the bacterial cell surface and the substrate. S. aureus adheres more readily to hydrophobic surfaces via numerous weakly binding macromolecules, while adhesion to hydrophilic surfaces involves fewer but stronger molecular interactions. These initial attachment mechanisms are crucial for biofilm establishment and stability on both biotic and abiotic surfaces. After microcolonies develop, an EPS forms and matures into a biofilm. Once the biofilm is fully grown, bacterial cells within it release compounds like D-amino acids and EPS-degrading enzymes (e.g., alginate lyase) to break down and disseminate the biofilm.
Concept of Biofilm (Lines 78-87)
The term "biofilm" describes a group of microorganisms embedded in a specific matrix that can adhere to inanimate and living surfaces. Biofilm growth on denture bases is identified as a primary cause of oral infections, particularly denture stomatitis. This illness affects 30% to 75% of denture wearers, manifesting as erythema on the palatal mucosa in direct contact with the prosthesis's fitting surface.
Challenges in Biofilm Treatment and Sustainable Innovations (Lines 88-95)
The manuscript highlights the difficulty in curing biofilms due to their complex, antibiotic-resistant nature and lack of indicators. This necessitates new material science solutions, especially for biofilm-resistant materials and traditional antibiotics. Sustainable innovations in antifouling are being explored, including targeting bacterial functions like quorum sensing, biofilm-related gene expression, secondary messengers, and regulatory RNA, as well as preventing initial adhesions with "green technology" like silicon oil-infused substrates from plant models.
Biofilm Composition (Lines 100-106)
S. aureus biofilm primarily consists of water (approximately 97%) and organic matter, including microcolonies and EPS. The main component of S. aureus biofilm EPS is polysaccharide-intercellular-adhesin (PIA), also known as poly-(1-6)-N-acetylglucosamine (PNAG). PIA's positive charge promotes colonization, biofilm formation, biofilm-based infections, immune system evasion, and resistance to antibiotics and phagocytosis.
Biofilms and Microbial Adhesion (Lines 107-146)
A biofilm is defined as a three-dimensional matrix formed by bacteria adhering to a surface by releasing insoluble gelatinous exopolymers, or a collection of extracellular materials and bacteria on a solid surface. From a medical perspective, biofilms, comprising both beneficial and harmful microorganisms, can attach to teeth or medical implants, embed in mucosal layers (bowels, lungs, vagina), or link to epithelial/endothelial linings. Biofilm formation and persistence have significant consequences for

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patients because microorganisms in biofilms are less vulnerable to topical treatments, antibiotics, and host defenses than their planktonic counterparts. Many biofilm infections are challenging to cure and often manifest as persistent or recurring. Biofilm infections lead to numerous clinical issues, including illnesses involving nonculturable species, persistent inflammation, poor wound healing, and rapid antibiotic resistance development.

Adhesion, where microorganisms securely cling to a surface through physicochemical interactions, involves an initial reversible physical contact followed by a time-dependent phase of irreversible chemical and cellular adhesion. Energy in the form of electrostatic, hydrophobic, and/or van der Waals forces is needed for microbes and surfaces to establish an adhesive connection. Bacterial adhesion, the first process of bacteria attaching directly to a surface, is more commonly referred to as adherence. The initial stage of bacterial adhesion, called attachment, is typically reversible and involves physical contact more than intricate chemical and cellular interactions. Microbial adhesion can be influenced by environmental factors such as temperature, exposure duration, microbial concentration, and the presence of antibiotics. For instance, the quantity of bacteria adhering to substrata surfaces increases over time until a saturation level is reached. These factors can affect bacterial adherence by altering physical interactions or the surface properties of bacteria or materials.

Microbes have developed biofilm formation as a survival strategy. Multiple microorganisms transition from a planktonic state to form intricate matrix-like structures as "communities". These dense micro-communities form on inert surfaces and encase themselves in secreted polymers. Organisms forming biofilms can adjust to environmental changes by altering gene expression patterns, which also shields them from antibiotics or disinfectants. The resulting biofilm can pose a major public health hazard.

Denture-associated biofilm microbiota also involves a regular group of microorganisms embedded in a matrix that adheres to living and nonliving surfaces. Biofilm growth on the denture base is a primary cause of oral infections like denture stomatitis, affecting 30% to 75% of denture wearers and manifesting as erythema on the palatal mucosa.

Factors Influencing Biofilm Development (Lines 151-172, Figure 1)

Several factors influence biofilm development:

* Surface roughness: Greater roughness increases microbial retention and biofilm biomass.
* Hydrophobicity: Hydrophobic interactions promote S. aureus adhesion to certain materials.
* Salivary pellicle formation: Salivary proteins can either enhance or reduce adhesion based on their composition.
* Material aging and wear: Long-term use alters surface topography and increases susceptibility.

Biofilms are implicated in epidemics, food spoilage, and equipment damage. A thorough understanding of factors influencing bacterial growth, such as attachment surface, surrounding circumstances, bacterial cells, and surface electrostatic charging, is essential. Environmental cues and bacterial extracellular surface elements are crucial for biofilm development and autoaggregation. Proteinaceous features like pili, fimbriae, lipopolysaccharides, and outer membrane proteins affect bacterial adhesion and autoaggregation phenotypes due to their advantageous positions on the cell surface.

Figure 1 visually summarizes the factors affecting biofilm formation, categorizing them into:

* Cell properties: Strain type, microbial growth phase, biofilm age, cell wall characteristics (Gr+ or Gr-), gene expression, presence of other bacteria (multi-species biofilms), autoaggregation/coaggregation, adhesion capacity, cell lysis, extracellular polymeric substances (exopolysaccharides, biofilm-associated proteins, extracellular lipids, extracellular DNA, extracellular RNA, uronic acid, humic acid, cations), metabolic activity, cell viability, population density, and cell adhesion molecules.
* Cell surface properties: Cell surface charge, flagella, pili, fimbriae and curli (cell surface appendages for motility), and hydrophobicity (cell surface physicochemical properties).
* Surface properties: Physicochemical factors (electrostatic forces, Lifshitz-Van der Waals, Brownian motion forces, Lewis acid-base interactions, hydrophobicity, functional groups, surface free energy,

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surface charge), surface chemistry/topography, surface geometry, carrier interface, and biomaterial types (roughness, smoothness, porosity, wettability, stiffness).

* Environmental conditions: Nutrient availability, water availability, flow rate of medium, temperature, incubation time, pH, salt, oxygen availability, relative humidity, ionic strength, hydrodynamic conditions, environmental signaling molecules, adhesins (receptor-ligand binding), exposure to antibiofilm agents, osmolarity, solutes, and quorum sensing.

Initial Attachment and Adherence (Lines 174-188)

Microorganisms typically adhere to surfaces quickly. Initial attachment in the biofilm life cycle occurs rapidly through physicochemical interactions between bacteria and the surface. Gene expression shifts quickly, and biofilm structure formation begins as the EPS physically attaches cells to the surface. Nonspecific adherence of microorganisms to surfaces involves both electrostatic and non-electrostatic interactions between the bacteria and the solid surface. Electrostatic forces are generated when electrostatic double layers with charged groups on either surface come into contact.

Material surface characteristics significantly influence microbial adherence to a biomaterial surface, including chemical composition, surface charge and hydrophobicity, and surface roughness. The attachment can be temporary or permanent depending on the interaction. Bacterial cells may form irreversible surface attachment using surface adhesins under environmental conditions. Adhesion to biotic surfaces usually requires a specific receptor-adhesin connection, while adhesion to abiotic surfaces is often mediated by nonspecific interactions. Biofilm formation and salivary protein adsorption/binding can affect the surface's hydrophobic/hydrophilic characteristics, surface energy, and availability of empty binding sites.

Denture Materials and Biofilm Formation

The manuscript then delves into specific denture materials and their susceptibility to biofilm formation:

* Acrylic Resin (Polymethyl methacrylate, PMMA) (Lines 195-206)

  * S. aureus preferentially sticks to polymers like PMMA, whereas Staphylococcus epidermidis prefers metals. This may explain why S. aureus is common in infections related to implanted metal medical devices, and S. epidermidis with polymeric devices. Bacterial adherence is prevented on surfaces altered with coatings like nonsteroidal anti-inflammatory drugs, antimicrobial peptides, or pluronic surfactants. PMMA is the most commonly used denture base material. Its porous and rough surface promotes microbial adhesion and biofilm maturation. S. aureus forms robust biofilms on untreated PMMA due to its hydrophobic nature and micro-porosities.

* Metal Alloys (e.g., Cobalt-Chromium) (Lines 207-233)

  * Surface roughness is a three-dimensional characteristic, often quantified by arithmetic average roughness (Ra). Terms like "surface finish" and "surface smoothness" are used interchangeably. Surface roughness significantly impacts microbial adhesion and subsequent biofilm formation.

  * This relationship is attributed to:

    * Initial bacterial adhesion in surface irregularities, shielding microorganisms from shear forces and allowing transition to irreversible attachment.

    * Increased surface area due to roughness, providing 2 to 3 times more sites for microbial attachment than smoother surfaces.

    * Cleaning challenges on rough surfaces, allowing residual cells to remain and facilitating rapid biofilm regrowth via cell multiplication rather than recolonization.

  * Bacterial adherence and retention are also influenced by surface free energy. Higher energy surfaces attract more plaque, bind it more firmly, and may favor specific bacterial species. However, surface roughness can have a greater impact than surface free energy. For example, increased roughness in older composite resin dental restorations due to wear and degradation processes is more likely to explain enhanced plaque buildup than changes in physicochemical surface characteristics.

* Thermoplastic Resins (e.g., Nylon-based flexible dentures) (Lines 235-264)

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* Removable partial dentures (RPDs) replace lost teeth to improve masticatory efficiency, phonetics, and prevent undesired tooth movement. The percentage of partial denture wearers is rising due to increased partially dentate adults, improved oral hygiene leading to a shift from complete to partial edentulism, and an aging population. Prosthetic materials in the oral cavity need specific properties. The denture base, typically made of metal or acrylic, shields soft tissue and supports prosthetic teeth. Polymer-based DBMs are prone to fracture, while metallic DBMs are heavy and technique-sensitive.
* An ideal DBM needs strength, durability, processing accuracy, dimensional stability, acceptable thermal characteristics, biocompatibility, high insolubility and low sorption in oral fluids, chemical stability, superior aesthetics, and ease of manufacture and cleaning. It should also adhere well to relining material and artificial teeth, be biocompatible with oral soft tissues, and be inexpensive and easy to repair.
* Thermoplastic resins show variable biofilm formation depending on composition and surface finish. Their flexibility can lead to micro-movements that promote microbial colonization in crevices. Material qualities for RPD denture base material are crucial for success. Nylon denture bases are a flexible alternative to PMMA in RPDs, aiding retention by forming a seal around the denture's edge.
* 3D-Printed Denture Resins (Lines 265-295)
* These are emerging materials with potential for improved smoothness and reduced porosity. However, surface irregularities can still support biofilm formation depending on the printing method and post-processing. Complete dentures remain the preferred treatment for edentulous patients with limited alternatives due to systemic illnesses, oral health issues, or financial constraints. Denture base resin (DBR) needs exceptional mechanical strength, stability, and biocompatibility for long-term durability and patient satisfaction. Combining 3D printing with nanoparticles can produce anodized nanosurfaces on medical implants with improved osseointegration and decreased polymerization, enhancing biocompatibility, durability, and cost-effectiveness. Research examines the potential antimicrobial effects of 3D technology and nanoengineering in dental/orthodontic implants, oral prostheses, joint replacements, hearing aids, catheters, stents, endotracheal tubes, prosthetics, and bone scaffolds.
* The use of 3D-printed reusable medical devices in various fields has led to safer medical equipment, reducing post-operative infections. Persistent infections from bacterial biofilm formation on implanted medical devices are a serious concern in healthcare. Bacterial infections are the most prevalent type of human infection, causing both acute and chronic conditions.
* Bacteria exist in sessile (surface-attached) and planktonic (free-floating) forms. In both phases, a protective barrier of exopolysaccharide matrix ("slime") is produced, making it harder for antibiotics to eradicate infection-causing bacteria.
* Zirconia and Ceramic Materials (Lines 296-333)
* These generally exhibit low S. aureus adherence due to high surface hardness and low porosity, and are more resistant to microbial colonization, but are less commonly used for full dentures. Data on the influence of electrostatic state and its connection to bacterial adherence for dental ceramics were not sufficiently demonstrated, but research suggests a relationship between topography, surface free energy, and bacterial adhesion.
* Zirconia dental implants are a game-changer as a biocompatible, aesthetically pleasing, and long-lasting alternative to titanium implants. Key characteristics include low bacterial affinity, superior aesthetics, and high fracture resistance. Zirconia's ability to osseointegrate with bone and its resistance to inflammation and plaque make it suitable for patients with high aesthetic standards or metal sensitivity. However, brittleness and complex manufacturing procedures remain challenges, with developments in surface modification and material optimization aiming to address these limitations.
* Zirconia dental implants are a viable titanium alternative due to improved biocompatibility, aesthetic benefits, and resistance to corrosion and bacterial biofilm production. Studies consistently show zirconia can lower inflammation and promote improved peri-implant health, making it a great option for those sensitive to metals or at risk of peri-implantitis. Its natural tooth-like appearance meets modern dentistry's

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cosmetic requirements, especially in the anterior region. Lower heat conductivity and corrosion resistance reinforce zirconia's potential as a durable and patient-friendly