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Original Article

Bacterial Biofilm in Chronic Suppurative Otitis Media

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Abstract

One of the most prevalent inflammatory lesions in the specialty of otorhinolaryngology is chronic suppurative otitis media (CSOM). A chronic suppurative inflammatory reaction of the middle ear mucosa and tympanic membrane, known as chronic suppurative otitis media, frequently invades middle ear tissues such the tympanum, mastoid process, tympanic sinus, and eustachian tube. Tympanic membrane perforation, hearing loss, and intermittent or persistent pus discharge in the ear are the most prevalent clinical signs of this illness.

Microbes living in tightly clustered, slowly expanding microcolonies and encased in a matrix made of a protective biopolymer form biofilm. The microorganisms develop the highest degrees of immunity to antibiotics and the immune system in biofilms. The middle ear cavity's bacterial biofilm may serve as a reservoir for the bacteria that cause recurring or chronic ear discharge in patients with CSOM. The benefits of controlling this form of infection, its impacts and complications, and preventing the emergence of antibiotic resistance may result from the successful elimination of such bacterial behavior.

Keywords: Chronic suppurative otitis media, Biofilm, Antibiotic resistance.

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Introduction

One of the most common pediatric diseases is still otitis media (OM), which comprises a wide range of disorders. Acute otitis media (AOM), recurrent acute otitis media (RAOM), otitis media with effusion (OME), chronic otitis media with effusion (COME), and chronic suppurative otitis media (CSOM) are all included in the spectrum . [(1)]

The significance of biofilm in persistent and recurring otorhinolaryngological infections has gained attention in recent years. An accumulation of bacteria in a matrix that has a high tolerance for antibiotics and the host defense is the key characteristic of a biofilm. The development of biofilm has been linked to the maintenance of the infection and a reduction in the effectiveness of antibiotic therapy.

This review presents an overview of chronic suppurative otitis media, its pathogenesis, bacterial biofilm development, and the current methods used to prevent, disperse, and treat bacterial biofilms.

Definition and types

Chronic suppurative otitis media is an inflammation of the middle ear cleft that manifests as recurrent ear discharge that lasts between six weeks and three months and comes from a rupture in the tympanic membrane. This syndrome can develop as a result of acute otitis media or as a result of retraction pocket formation and eustachian tube dysfunction. As a

result of a spontaneous tympanic membrane perforation, the pathogenesis may begin in childhood. [2]

Chronic suppurative otitis media can be categorized into two types; **benign CSOM** (safe type) or commonly called tubo-tympanic type, associated with permanent central perforation, which does not cause serious complications, and **malignant CSOM**, also called attico-antral type. This type is associated with ossicular erosion due to cholesteatoma, granulation tissue, or osteitis. Complications that arise from malignant CSOM are quite dangerous . [3]

Middle ear cholesteatoma (MEC) is the term for an abnormal accumulation of squamous epithelial cells in the tympanic cavity, tympanic sinus, mastoid cavity, or connective tissue underneath the epithelium as well as the accumulation of keratinized fragments with or without an inflammatory response to the surrounding area. [4] Bacterial infections develop due to cholesteatoma's expansively expanding epidermal masses . [5]

Pathogenesis and etiology

Genetic, environmental, and eustachian tube (ET) anatomical and functional traits are all part of the multifactorial pathophysiology of CSOM(6).Even if the relationship between acute and chronic otitis media may seem uncertain the development of CSOM is typically insidious . [7]

Frequent episodes of acute otitis media, various respiratory tract infections, and traumatic tympanic rupture are risk factors for CSOM. Living situations with inadequate resources, such as overcrowding, poor nutrition, and hygiene, as well as persistent infectious diseases, are other contributing factors. [8]

Children with eustachian tube dysfunction

(as in cases of cleft palate and Down's syndrome, as well as a patulous ET allowing nasopharyngeal reflux), mucosal immune system (MIS) deficiencies (specific to URT mucosa immunity and atopy), systemic immune deficiency (abnormalities of humoral immune system and cell-mediated immunity), and inadequate mastoid pneumatization are among the patients who are most susceptible to CSOM. [7]

The microbiota identified in chronic suppurative otitis media (CSOM) include potential pathogens such as aerobes, anaerobes, and fungi. Nonetheless, the reported profile and frequency of these microorganisms vary depending on the patient's age, location, and the presence of complications like cholesteatoma.[9]

A polymicrobial etiology is common. Staphylococcus aureus (MRSA) is the most frequent microbial species identified in this condition. Other pathogens that can cause the disease include Pseudomonas aeruginosa, Proteus species, Klebsiella species, Bacteroides species, and Fusobacterium species (10). Aspergillus spp. and Candida spp. are less common microbes that are more usually detected in immunocompromised people (10).Chronic suppurative otitis media can also be caused by tuberculosis; however, it is more typical in regions where the disease is prevalent . [11]

Otorrhea and hearing loss are the two primary symptoms associated with chronic suppurative otitis media. Cholesteatomas frequently progress slowly before becoming invasive and symptomatic. Cholesteatoma can occasionally cause a sudden intratemporal or intracranial complications. A cholesteatoma may first manifests symptoms of facial palsy. Mastoiditis, petrositis, labyrinthitis, facial nerve paresis, and labyrinthine fistula are examples of intra-temporal problems. [12] Abscess or extradural granulation tissue, brain abscess, sigmoid sinus thrombophlebitis, otic hydrocephalus, meningitis, and subdural abscess are among the intracranial sequelae.[12]

Biofilms

Extracellular polymeric substance (EPS), which includes biomolecules such lipopolysaccharides, lipids, polysaccharides, proteins, and deoxyribonucleic acid (DNA), serves as a protective matrix for populations of bacteria called biofilms. [13]

According to Verderosa et al., biofilms are intricate, three-dimensional communities of microorganisms that are attached to a surface and covered in an extracellular polymeric substance (EPS) for protection. The primary source of nutrients within a biofilm matrix is the extracellular polymeric material, which is made up primarily of water (up to 97%), polysaccharides (1%), RNA, and protein (1%), respectively. [14]

Staphylococcus epidermidis, Staphylococcus aureus, P. aeruginosa, and Enterobacteriaceae are the most prevalent microorganisms, but all bacteria are

capable of forming bacterial aggregates of a size and structure that confer biofilm properties in the presence of a host predisposing factor. (15)

The reversible attachment to a surface via certain interactions between the bacterial wall and the substrate is the initial stage of biofilm formation. The microorganism is prompted by the interaction to synthesize and release extracellular matrix distant surfaces .(16)

components as well as to reinforce the reversible cell-substrate linkages. The matrix enables other species to adhere to the growing colony as it develops a mushroom-like morphology. When the biofilm reaches maturity, it has the ability to release some of its colonies into the environment, to further colonize

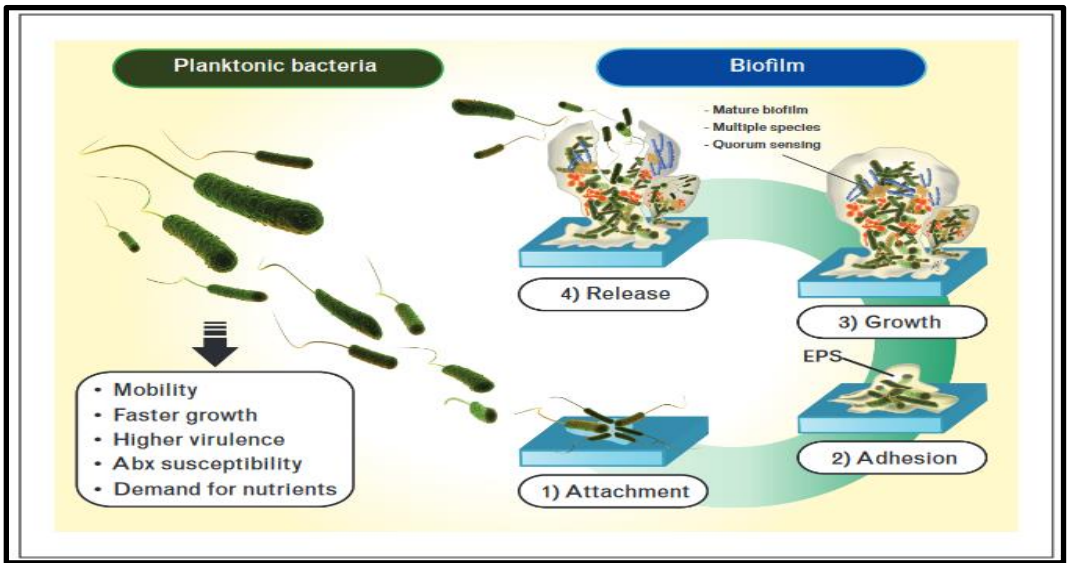

Figure (1): Biofilm formation. (16).

Antimicrobial tolerance and resistance:

Tolerance and resistance are the two dimensions of the decreasing sensitivity of bacterial biofilms to antimicrobial treatments. Although bacteria within a biofilm typically survive antibiotic treatment, they become vulnerable to the therapy when the biofilm is broken, therefore biofilm antibiotic tolerance should not be mistaken with antibiotic resistance. (17)

In contrast to resistance, which permits bacteria to proliferate in the presence of antibiotics, tolerance means that bacteria are not killed despite the fact they are unable to grow in the presence of the drug. When a certain quantity or density of bacteria has accumulated, tolerance may result, whereas resistance will gradually increase owing to internal and external factors like mutations . (18)

Antibiotic-modifying enzymes such β-lactamases, which can inactivate antibiotics before they reach bacterial cells and are abundant in the outer layers of the biofilm, can be found in the matrix in both Gram-negative and Gram-positive bacteria. (19)

Contrary to tolerance, antimicrobial resistance is not temporary, persists in bacteria even after a biofilm has been disturbed, and is brought on by either bacterial genome changes or the acquisition of antimicrobial resistance genes by horizontal gene transfer (HGT).(20)

The maintenance of a large number of bacterial cells that survive antibiotic treatment due to the tolerance of the slow-growing population, the presence of persisters, and a high mutation rate are among the many factors encountered in biofilms that contribute to the development of antibiotic resistance. (20)

Persister cells can withstand high dosages of antibiotics because they lack genetic resistance determinants. (21) Persister cells are thought to be non-dividing cells that may transform back into rapidly proliferating cells when nutritional scarcity conditions or antibiotic treatments are lifted, resulting in a recurrence of the biofilm infection. (22)

Due to its capacity to enhance pathogen survival

and encourage unfavorable host-bacteria interactions, biofilm has been referred to as an "influencer of infections". [23]

The matrix exopolysaccharide polysaccharide synthesis locus (Psl) in ***P. aeruginosa*** guards against opsonization and complement system killing of biofilms. [24] In biofilms, ***S. aureus*** secretes immune-evasion molecules that impair opsonization and the complement system. [25] Despite allowing phagocytosis, the anaerobic environment around the biofilms prevents the death of bacteria, which requires an oxygen-dependent respiratory burst that produces reactive oxygen species (ROS).[26]

Bacterial quorum sensing:

A method of bacterial cell-to-cell communication known as quorum sensing (QS) enables bacterial communities to exchange information concerning their changing environment by producing, perceiving, and responding to extracellular signaling molecules .[27] Bacteria that use quorum sensing can interact by using signaling molecules known as autoinducers (AIs).

Quorum sensing controls a number of essential bacterial survival mechanisms and enables bacteria to adapt to variations in cell density. [28] These include the formation of biofilms, the release of virulence factors, bioluminescence, motility, the production of antibiotics, sporulation, and the growth of genetic competence [29]

Biofilm detection and elimination:

The method of biofilm detection that is used depends on the location of the biofilm's formation and the sample being examined. The guideline recommends some detection techniques for biofilm laboratory diagnosis, including polymerase chain reaction (PCR) and electron microscopy, where it is possible to recognize microbial aggregations around inflammatory cells. [30]

The best method to find biofilms is considered to be a biopsy. [31] The extracellular matrix of the biofilm, immune system cells, and microbial aggregates are all visible in stained biopsy samples. [30] Biopsies are frequently not possible in clinical settings; instead, other samples, such as sputum, blood, fluids, and secretions, may be sent to the laboratory. [32]

Since the microorganisms in the biofilm are adherent to one another and a surface, forming a microbial aggregate, laboratory examination of these samples is challenging. As a result, sonication is typically used to clear biomaterial surfaces of the gathered and adhering bacteria. [33]

Following sonication, the aggregated bacteria separate from the material's surface and can be examined usinga variety of techniques, such as the polymerase chain reaction, which is utilized for the direct detection of pathogens that form biofilms in clinical samples. This technique is based on the amplification of specific sections and offers great specificity and sensitivity in the identification of genes involved in biofilm formation [34]

Fluorescence in situ hybridization (FISH) is a technique that involves adding short, fluorescently-labeled oligonucleotides that can bind to a particular target organism's ribosomal RNA. Microscopy is used to assess the samples. [35]

Electronic microscopy; using this technique, microbial aggregates that have undergone sonication or FISH can be seen. The most popular methods for seeing biofilms are scanning electron microscopy (SEM), transmission electron microscopy (TEM), and confocal laser scanning microscopy (CLSM). Samples can be fixed, dehydrated, and stained using SEM, but throughout each of these steps, especially the dehydration step, the morphology and structure of the biofilm may alter, which is a drawback of the method. [36]

The biology of biofilms and their detection has been better understood via the application of numerous in vitro and in vivo techniques. Congo red agar test: Because it is based on a subjective chromatic assessment, this test is quantitative .[37] Despite being an inexpensive test, the tube biofilm formation test is qualitative and subjective. [38]

Microplate test: In this test, flat-bottomed or U-shaped microplates are used, and all stages of biofilm formation take place inside these wells. The wells containing the samples are dyed with particular dyes to measure the primary structures of the biofilm in order to detect biofilm formation. The 'colorimetric test' is what this portion of the experiment is known as [39].

Each isolate in these colorimetric assays can be categorized into nonproducers, weak, moderate, or strong producers of biofilms based on the absorbance results. However, this categorization is incredibly subjective and can change depending on the species and the reference cut-off value that the researcher employs. (40)

The idea that biofilms offer persister cells a "safe haven" to develop and resist antibiotics and immunological components leads to the conclusion that in order to successfully eradicate pathogens from infection sites, whole biofilm structures must be removed. The preferred method of managing chronic wounds is mechanical debridement (scraping biofilms from wounds). (41)

Chemicals that promote biofilm dispersion have been used alone or in conjunction with antibiotic therapy in cases of internal biofilms. Examples include Dispersin-B, that degrades a common biofilm matrix component called poly-N-acetylglucosamine (42)

Even with medicinal therapy to the underlying infection, to which primary pathogen, *P. aeruginosa*, is sensitive, otorrhea and inflammation cannot be reduced in refractory COM. In addition to its other advantages, N-acetyl cysteine (NAC) also has an eliminating impact on the biofilm layers formed by various bacterial species. (43)

Additionally, bacteriostatic, antibacterial, and antibiofilm properties of boric acid have been discovered. As a result, for recurrent or chronic inf-ections, boric acid may be used alone or in conjun-ction with another antibiotic. According to a study, treating CSOM with a direct application of boric acid powder to the external ear canal (EAC) after flushing it with saline produced positive results. (44)

Boric acid is reasonably successful for treating CSOM, especially when given at high dosages, according to *Adriztina et al.* It may be used both separately and in conjunction with antibiotics. Boric acid administration is expected to stop the overuse of antibiotics that can result in microbial resistance. (45)

Conclusion:

One of the conditions that otorhinolaryngologists see the most commonly is chronic suppurative otitis media (CSOM). Chronic middle ear and mastoid cavity inflammation may manifest as at least two-weeks-long recurrent ear discharges from a tympanic perforation. It is a problem for public health in developing countries. Different microorganisms that damage the middle ear cleft can cause chronic suppurative otitis media. The two main microorganisms are Staphylococcus aureus and Pseudomonas aeruginosa. Biofilms may contribute to the CSOM's persistence, recurrence, and challenging eradication.

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