Otitis Media and Biofilm: An Overview

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Abstract

Otitis media (OM) is a group of complex infective and inflammatory conditions affecting the middle ear, with a variety of subtypes differing in presentation and associated complications. It is the highest cause of pediatric health-care visits and antibiotic prescriptions internationally. It has been stated that approximately 75% of children under age three suffer from middle-ear infections. OM complications are the essential cause of preventable hearing loss, particularly in low-resource settings. Currently, scientists have confirmed that bacteria form colonies in the middle ear and are responsible for chronic infections. While bacteria are often thought of as independently free-floating living microorganisms, most of the bacteria and fungus forms organize complex communities and attach to surfaces called biofilm. Biofilm formation is considered as a survival strategy by bacteria to counteract traditional approaches (such as antibiotics) which are effective against bacteria. Biofilms are almost impossible to grow in the laboratory media and are incredibly resistant to antimicrobials which mean that the diagnosis of chronic OM is one of the most challenging in the management of middle-ear infection. This article aims to provide an appraisal of current scientific successes within the field of OM research and clinical management.

Keywords: Biofilm, otitis media, treatment strategies

INTRODUCTION

Otitis media (OM) is a standard terminology [1] clinically comprising acute OM (AOM), chronic OM (COM), and COM with effusion (COME), also referred to as nonsuppurative OM. [2,3] OM is a common middle-ear pathological condition in both high- and low-resource settings, a principal cause for visiting the doctor (particularly for pediatric patients), requires antimicrobials, surgical procedures, and is the primary cause of hearing loss. [4-6] COM is associated with chronic suppurative OM (CSOM) but is different as COM is not necessarily a consequence of “the gathering of pus.” [7] Streptococcus pneumoniae, Haemophilus influenzae, and Moraxella catarrhalis have been isolated from approximately 25% of children with OME and polymerase chain reaction (PCR)-based methods have demonstrated sequence-specific DNA and RNA for these pathogens in nearly 80% of cases. This finding specifically relates to OM with effusion, lasting for more than 3 months with an intact eardrum. [8-13]

CHRONIC SUPPURATIVE OTITIS MEDIA

CSOM is considered as the most prevalent chronic contagious disease of prepubescence and adolescence in many low-resource countries and among marginalized communities (especially among aboriginal and indigenous peoples living in Australia and Alaska, United States, respectively) in developed nations and is a major cause of acquired hearing impairment affecting 65–330 million people globally. [14,19-23] CSOM is “probably a dangerous clinical condition” [24] which can lead to deadly intracranial infections and acute mastoiditis, particularly in resource-poor countries and is a cause of poor academic achievement. [25] CSOM is defined as a chronic persistent inflammatory multifactorial disease of the middle ear and mastoid cavity, which presents with recurrent and persistent ear discharges (or otorrhea) through a tympanic membrane perforation (TMP) for over 2–6 weeks. [25-28] CSOM can cause thickening of the middle-ear mucosa, mucosal polyps, and cholesteatoma. [29]

The classification and definition of CSOM have changed over time. Conventionally, it was classified as a safe or tubotympanic...
Bacteria are commonly involved in biofilm formation.[49,42-44] This has been recognized as the most important causative factor of the persistent nature of COM.[61,62] Biofilms have now been studied in other middle-ear diseases of children, such as recurrent AOM with middle-ear effusions, which increases the possibility of COM.[48,63-65] Bacterial biofilms were found in 85%, 92%, and 16% of patients with middle-ear cholesteatoma, COM, and TMD, respectively.[57] Another factor associated with COM is the presence of biofilms, which explains the lack of antibiotic efficacy for this disorder.

Although there have been technological advances in laboratory diagnostics, routine clinical, microbiological examinations continue to depend on more traditional testing methods (including culture, phenotypic, and biochemical tests) to detect microorganisms present in the clinical specimen.[66] Among these, diagnostic test culture and sensitivity is one of the definitive diagnostic tests in microbiology, and the plate count technique is one of the standard cultivation methods for the itemizing and quantification of viable bacteria.[67,68] This traditional diagnostics approach is important, reliable, and makes a huge contribution toward the diagnosis of infectious diseases and treatment; however, it is less specific for biofilm detection because these methods cannot identify the composite, three-dimensional features of biofilms.[69-71] Staphylococcus species, Streptococcus species, Pseudomonas aeruginosa, and other bacteria are grown at experimental conditions (usually 37°C for 24–48 h). However, most bacterial and fungal species do not grow under these laboratory conditions. Bacteria in the biofilm phenotype are especially difficult to grow in routine clinical microbiological culture examinations because these organisms are viable but not culturable (VBNC) state.[72-74]

A group of scientists first observed in 1982 that Escherichia coli and Vibrio cholerae cells could enter a distinct state called the VBNC “nonrecoverable stage of existence.”[74] A large proportion of the biofilm community of pathogenic bacteria continues as a VBNC state, and traditional diagnostic techniques fail to detect these organisms. The VBNC state Staphylococcus epidermidis present in biofilms are not only
resistant to several antimicrobials but also escapes normal defense mechanisms. In vitro decreases the output “of tumor necrosis factor alpha, interleukin-1, and interleukin-6 by bone-marrow-derived murine macrophages, and in vivo, a lower stimulatory effect on peritoneal macrophages, assessed by increased surface expression of Gr1 and major histocompatibility complex class II molecules.”[75] Normally, bacterial cells are culturable inappropriate media, and they develop their colonies in a culture plate. VBNBC bacterial cells are alive and viable cells which have lost the ability to grow in routine laboratory media, on which they normally grow.[76] Although biofilm bacterial cells have lost the potential to grow typically in compliant laboratory media, VBNBC cells are not considered as dead. Whereas, the cell membrane of dead cells is totally damaged, incapable of holding chromosomes and plasmidic DNA, and metabolically inactive, VBNBC cells have a complete and integral cell membrane, comprise intact genetic information, retained plasmids, maintains metabolism, and carry out respiration.[77-81] Furthermore, dead cells do not express genes, whereas VBNBC cells endure transcription and consequently, mRNA production.[73] Dead cells do not need or utilize nutrients, in contrast, VBNBC cells are known to have continual uptake and integration of amino acids into proteins.[82] For example, the VBNBC state *Listeria monocytogenes* had high levels of ATP even after 1 year.[83] The VBNBC “state is, therefore, a unique survival strategy adopted by many bacteria in response to adverse environmental conditions.”[84] Multiple studies have revealed that over 50% of middle-ear effusion specimens were found to be culture negative.[48,58-89] This raises suspicions that microorganisms are not involved in OME.

Molecular biology diagnostics technology based on nucleic acid intensification approaches have provided the process to detect and recognize bacteria, and different imaging modalities have given investigators insight into the role of biofilms in human infections.[86] Scanning electron microscopy is also a cutting-edge resolution technique that makes available ultrastructure analysis of biofilms.[89] Studies using PCR tests have found mixed results. Some studies detected bacterial nucleic acids in a clinical specimen of middle effusion of several patients.[15,91,92] However, another study concluded that nucleic acids noted through PCR evaluation might not denote viable bacteria, even when the quantitative technique of real-time PCR was used,[93] although an innovative technique of distinguishing whether DNA comes from live or dead bacteria has newly been developed.[94] An alternative interpretation is that the nucleic acid detected might be VBNBC not culturable by traditional laboratory approach, a state that is usually observed in biofilms.[95,96] More evidence that biofilms are involved has been found, for example, one reporting that bacterial biofilm is progressively documented as the causative factor for the recurrence and persistence of infections such as OM.[90] Multiple live otopathogenic species were demonstrated in bacterial biofilms of OME of children with recurrent acute OM (RAOM). There was also the association of these biofilms with a DNA matrix produced by neutrophil extracellular traps.[96] Furthermore, bacterial biofilms were confirmed in 84% of OME from children undergoing ventilation tube insertion for RAOM.[84] Similar rates of the presence of biofilm on the middle-ear mucosa among pediatric patients with COME and RAOM (64%–92%)[48,63] and in COM discharge samples (83%) in earlier studies were found.[97] Utilizing fluorescent in situ hybridization technology, these biofilms of OME were found as heterogeneous, polymicrobial community, and consistent with the environment.[98,99]

One of the exclusive characteristics of biofilms is “planktonic shedding” of bacteria from the biofilm surface into the neighboring space, scattering infection to distant parts of the body in a manner like septic emboli.[100-102] This planktonic shedding is a regular event which appears to intensify during physiological stress and starvation.[103] The planktonic-tattered bacteria are consistent with common pathogens found in nasopharyngeal biofilms and OME of children with RAOM and chronic OME. Therefore it has been concluded that there is a hidden pathogenesis of RAOM and OME with high rate of negative findings in the middle-ear fluid OME cultures and common recurrence of these diseases.[103-106]

**TREATMENT STRATEGIES OF BIOFILM-RELATED MIDDLE-EAR DISEASES**

Multiple studies report that biofilm chronic infective disorders are difficult to eradicate and often antibiotic treatment alone is inadequate.[69,107-109] In general, the treatment strategies can be divided into three main types based on the involvement of a foreign body (device) or not.[85,103,110] Those are device-related biofilm disease, nondevice-related chronic biofilm disease, and biofilm-related device malfunction.[107] “Implanted foreign bodies are highly susceptible to pyogenic infections and represent a major problem in modern medicine.”[111]

**DEVICE-RELATED BIOFILM DISEASE**

Medical implant-related infectious diseases are often very serious and increase morbidity, mortality, and health-care costs tremendously.[112-114] The average rate of surgical implant-related infectious diseases ranges between 2% and 40%. [112,113] Earlier studies demonstrated that *Staphylococcus aureus* inoculated guine pig modeled soft tissues could not create any abscesses in the absence of foreign body, whereas infection and inflammation could be induced with a foreign body in 95% of the cases, despite a significant presence of polymorphonuclear (PMN)/[granular] neutrophil, eosinophil, and basophil) leukocytes.[111,115] The presence of a foreign body significantly decreases the phagocytosis and intracellular bactericidal effects of PMN leukocytes.[111] Due to reduced granular leukocyte functions, a foreign body (device/implant) provides a perfect surface for microbes to attach.[69] Therefore, a foreign body increases the predisposition significantly for the possibility of biofilm formation[69] and infection to staphylococcal infection by at least 10,000 fold.[111,112,116] Consequently, the removal of a foreign body is an important
measure to help eliminate such biofilm infections.\[69,117,118\] The most important first step in treating a biofilm infection is to have a high index of suspicion. The mainstay of treatment includes antiseptic techniques, removal of infected foreign bodies, and provision of meticulous debridement. However, with an understanding of biofilms and how bacteria interact to form biofilms, newer methods can be developed in the future.

It is possible that not all medical devices or implants (foreign bodies) progress to chronic biofilm-related infections, and biofilms are also observed in the absence of such implant.\[107,109,119-122\] In view of this, possible antibiofilms should be constructed around the three-stage process of biofilm formation: “(i) Inhibition of microbial adhesion to the surface and of colonization, (ii) interference with the signal molecules modulating biofilm development, and (iii) disaggregation of the biofilm matrix.”\[117\] Another group of scientists has categorized antibiofilm research and the development of intervention technologies into two groups: (a) explicitly prevents the formation process of biofilm and (b) the development of medical devices or implants that will be resistant to biofilm formation.\[117\] In the last two decades, although great scientific and technological advances have been achieved regarding medical implants that are resistant to microbial colonization and biofilm formation, still most of the targets of biofilm remain the same and they continue to tax human health care.\[122\]

That bacteria could stick to, survive, and proliferate on surfaces was first documented in a scientific medical journal in 1937.\[123\] Another group of researchers found natural populations of bacteria (predominantly Gram-negative) enmeshed in a fibrous matrix whose constituent fibrils were tarnished with ruthenium red, with slippery, mucoid, and muddy submerged surfaces in a mountain stream.\[124\] The surface is the most important factor in biofilm formation. The preliminary add-on and fixing of microbes to surfaces are controlled by physicochemical properties.\[125\] The universal rule of thumb is that microbes will favorably inhabit surfaces that are hydrophobic, have even a minor degree of coarseness, and are exposed to a conditioning layer in distinction to smooth, hydrophilic surfaces. Research confirms that the principal difficulty is to prevent the formation of a conditioning layer that provides microbes with a place to lodge and form a colony.\[126\] Almost all substances in natural environmental conditions, particularly when exposed to air and humidity, are subjected to microbial contaminations. Researchers are exploring several mechanisms aimed at developing coatings that are either antimicrobial or nonbiofuling.\[125,127\]

Another group of scientists reports that on polymethacrylate derivate painted surfaces where microbes attached after 1 h of exposure, 99.9% were dead. Over the next 2–8 days as the painted coating slowly hydrolyzed, 98% of the microbial cells were released.\[128,129\] The polymethacrylate derivate with a cationic side chain becomes zwitterionic on the conversion of a terminal ester to carboxylate, and the hydrolysis-painted coating prevents further attachments of microbial cells and formation of a biofilm. Several researchers are exploring various avenues of research on a range of medical devices used as implants.\[128,129\] Polycationic polymers have been extensively utilized as antimicrobials because of their broad-spectrum activity against both bacteria (E. coli and S. aureus) and fungi (Candida albicans). Furthermore, such polyanionic antimicrobial membranes competitively prevent the development of biofilms by SC5314 and its crk1 gene deleted (Δcrk1) C. albicans strains.\[130\]

**LIMITING MICROBIAL ADAPTABILITY**

Microbes essentially possess an ability to sense their environments and can adapt their physiological systems to acclimatize and continue to survive better.\[69\] This ability of microbes (bacteria and fungi), global regulation, is considered as the most important function, specifically quorum sensing (QS).\[119\] Microbes use QS to coordinate gene expression in response to fluctuations in cell-population density. This functions as a decision-making process to regulate the production of virulent factors and create an infection.\[131-134\]

In the last decade, it has been identified that nucleotide signaling controls many of the vital processes required for microbial adaptation and is associated with pathogenicity as QS. Among the above, several nucleotides (cyclic diguanosine monophosphate [c-di-GMP], cyclic diadenosine monophosphate, cyclic guanosine monophosphate, cyclic adenosine monophosphate and guanosine tetraphosphate, and c-di-GMP [The Universal Bacterial Second Messenger]) have been under active consideration since they are involved closely in biofilm formation in Gram-negative bacteria.\[135-138\] Consequently, the control of QS mechanism and the alteration of c-di-GMP become the focus of the development of new antibiofilm medicines. Parallel to QS and c-di-GMP, microbial amyloids have spawned another widespread area of new research.\[69\] Amyloids have been identified in several types of microbial species which rely on amyloids to stick to one other or to further host surfaces, resulting in the creation of biofilms.\[139,140\] Medicines which damage amyloid structures might provide an innovative means of preventing and eradicating microbial biofilm-related infectious diseases.\[141\]

The commonly used antimicrobials achieved wonderful success in eradicating infective disorders both in hospitals and the community.\[59,142\] However, they have not been able to generate promising results regarding biofilm diseases because biofilm cells are at least 500–1000 times more resistant to antibacterial agents,\[106,125\] as biofilm microbes reduce rates of cellular growth, respiration, metabolism, and protect themselves by biofilm matrix polymers.\[143,147\] The expression of such precise defensive aspects, such as multidrug efflux pumps and stress-response regulons, additionally increased biofilm resistance against antimicrobials\[146-153\] as well as plasmidial gene transfer, which is enabled in the biofilm setting.\[154\] Furthermore, the heterogeneity in metabolic and reproductive commotion within a biofilm is associated with an erratic susceptibility of fenced bacteria.\[150\] Thereafter,
Multiple groups of otolaryngologists concluded that multiple strategies are involved in the development and maintenance of biofilms resistance against antimicrobials [55, 56, 155-157]. Consequently, present-day ear-nose-and-throat specialists are facing the spread of biofilm-related infections, although the concept of biofilms is a relatively recent issue to many otorhinolaryngologists [158]. Many scientists feel that there is an urgent need among ENT surgeons to develop a better understanding of pathogenesis biofilms and their contribution to biofilm-related ENT diseases that will hopefully trigger the progress of novel treatment strategies [156, 158].

Genetic and Preventive Strategies

The genetic factors subsequent to indisposition to OM are not well understood although a few genetic targets have been recognized [159]. Some studies report that it has been documented in reputed journal concerning a familial connection of AOM and COM as 40%–70%, but most of the genes underlying this susceptibility are yet to be identified [159-163]. It is suggested that the innate defense molecules become defective, leading to increased susceptibility to OM [164-167]. Possible therapeutic goals, therefore, aim to identify and target the genes regulating mucin expression, mucus production, and host response to bacteria in the middle ear [165, 166]. Comprehensive understanding of the genetics of OM is of absolute necessity for the development of anticipatory actions, and the minimization of risk aspects in susceptible patients. Research in mouse models regarding OM found that hypoxia is as an essential factor in OME, and as ventilation tubes, implant relieves hypoxia and reverse the disease process of the middle-ear bacterial and viral infection [165, 167].

It has been considered that defensive measures for OM and its most dangerous complication COM are the most important in limiting the impact of this disease [159]. The most common invading organisms for AOM are S. pneumoniae, nontypeable H. influenzae, and M. catarrhalis [14, 168]. Antigens against S. pneumoniae, nontypeable H. influenzae, and M. catarrhalis have been identified and shed light on the prevention of middle-ear diseases by the development of a vaccine [1168]. Pneumococcal conjugate vaccine (PCV7) is highly immunogenic in children in the first few months of life and includes most of the serotypes associated with AOM (4, 6B, 9V, 14, 19F, 18C, and 23F). As S. pneumoniae is the cause of 25%–50% of the cases of AOM, and PCV7 prevented more than 90% of the cases of invasive pneumococcal disease, it was thought that a significant reduction in the incidence of AOM might follow the implementation of programs for its universal use in younger children [38].

One systematic review concluded that OM visit rates have decreased by approximately 19% following the introduction of the 7-valent pneumococcal conjugate vaccine [1169]. Another study reported a 12% (95% confidence interval [CI] 0.5–26) decline in the number of AOM incidents caused by S. pneumoniae in patients <3 years between 2000 and 2007 due to the combined effects of PCV7 [a heptavalent vaccine, meaning that it contains the cell capsule sugars of seven serotypes of the bacteria S. pneumoniae (4, 6B, 9V, 14, 18C, 19F, and 23F)] vaccine efficacy and vaccine-induced serotype replacement, and furthermore, expects that PCV13 (pneumococcal conjugate vaccine called PCV13) defenses against 13 types of pneumococcal bacteria will further decrease pneumococcal AOM an additional 27% (95% CI 13–40) from 2007 to 2013 [170]. Another systematic review of 11 randomized controlled trials, in which 46,074 children were given a pneumococcal vaccination for the prevention of AOM concluded that a reasonable result of pneumococcal polysaccharide vaccination was found in children of 2 years and more (relative risk [RR] 0.78; 95% CI 0.63–0.97). Pneumococcal polysaccharide vaccine had little outcome on prevention of AOM in children without earlier recorded episodes before vaccination (RR 0.92; 95% CI 0.85–0.99). Improved usefulness was seen in children with documented previous AOM before immunization (RR 0.81; 95% CI 0.72–0.91). Collective outcomes of pneumococcal conjugate vaccine trials in infants vaccinated as early as 2 months of age and in toddlers attending day care showed a limited effect on the prevention of AOM (RR 0.92; 95% CI 0.85–0.99). Other research concluded, based on current evidence of the efficiency of PCVs for the deterrence of AOM, that licensed 7-valent PCV given during infancy has wide-ranging useful effects. The study found that 6%–7% reductions of the middle-ear diseases might be considered as important from a public health standpoint. However, PCV7 vaccine given in older children with a history of AOM appears to have no advantage in deterring added episodes [172]. Two other studies reported almost similar findings regarding the licensed 7-valent PCV with CRM197 as a carrier protein (CRM197-PCV7) [173, 174].

Conclusions

Biofilm-related middle-ear diseases ranging from AOM to COM still continue to affect many children worldwide, increasing both morbidity and mortality. Although more in-depth understanding of biofilms is needed to prevent middle-ear diseases, a range of scientific developments are providing new ways of identifying biofilms and limiting their impact, including the development of new materials for implants and devices, medications, targeted gene therapy, and vaccinations [23].

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Conflicts of interest

There are no conflicts of interest.
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