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The Role of Toll-Like Receptors and Nuclear Factor κB p65 Protein in the Pathogenesis of Otitis Media

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Abstract

The role of Toll-like receptor 4 (TLR4) and nuclear factor κB p65 (NF-κB p65) proteins in the pathogenesis of otitis media is explored. In recent years, the incidence of otitis media has been rising globally, becoming a significant threat to human health. More and more studies have found that Toll-like receptor 4 (TLR4), as a member of the Toll-like receptor family, can promote the generation of inflammatory factors and is closely related to the body's immune response and inflammatory response. Nuclear factor-κB p65 (NF-κB p65) is a nuclear transcription factor that can interact with various cytokines, growth factors, and apoptotic factors, participating in processes such as oxidative stress, apoptosis, and inflammation in the body [1]. This article elaborates on the structure, function, and signaling pathways of TLR4 and NF-κB p65 proteins in the pathogenesis of otitis media, aiming to provide more precise targets and better therapeutic efficacy for the diagnosis and treatment of otitis media. The role of inflammation in disease.

Keywords

Otitis Media, Toll-Like Receptors, Nuclear Factor κB p65, Signaling Pathway

1. Introduction

Otitis media (OM) is considered one of the most common diseases in otolaryngology worldwide, with an estimated incidence of 1.7 - 9.37 per 1000 people, depending on the region of the world [2]. The disease can occur at any age, with clinical symptoms mainly manifesting as recurrent ear discharge, tympanic membrane perforation, hearing loss, and tinnitus. Hearing loss is primarily caused by conductive hearing loss due to damage to the middle ear's sound transmission

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structures, but it can also manifest as sensorineural hearing loss caused by pathogens damaging the cochlea. This affects speech, language, and cognitive development. In severe cases or when treatment is inadequate, the lesions may invade the surrounding bony structures of Complications may include mastoiditis, cholesteatoma, meningitis, brain abscess, and cavernous sinus thrombophlebitis; recurrent infections and persistent treatment challenges have always been a difficulty faced by clinical practitioners [3] [4]. A large number of studies have shown that the occurrence and development of otitis media are closely related to various inflammatory factors acting through different signaling pathways. Therefore, reducing the production of inflammatory factors can serve as a starting point for treating various acute and chronic inflammatory diseases. In recent years, the mechanisms underlying the inflammatory response.

2. Common Pathogens of Otitis Media

The risk factors for otitis media (OM) include gender, family genetic predisposition, environment, adaptability and natural immune system, eustachian tube dysfunction, and previous or concurrent bacterial and viral infections, among others, with infections being the most common cause of otitis media. The common pathogens in acute otitis media are *Streptococcus pneumoniae*, *Haemophilus influenzae*, and Catamoeba, and in chronic suppurative otitis media (CSOM) are *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Escherichia coli*, and anaerobic organisms such as *Bacillus* spp. and *Clostridium* spp. [4]-[6]. There is a strong relationship between viral upper respiratory infections (URIs) and acute OM (AOM), with the development of AOM requiring a pre-existing viral URI, and this link has been strengthened by recent studies showing that during viral epidemics there is an increase in AOM, and that rhinoviruses are the most common respiratory viruses causing URIs, and are thought to play an important role in the development of AOM [7]. In addition, fungal infections such as *Aspergillus* spp. and *Candida* spp. can also contribute to the progression of the disease in areas with specific geographic conditions or in individuals with a weakened immune system, which has been linked to humid environments due to prolonged ear canal drainage in conjunction with prolonged antibiotic use [8]. The research report by Qiulin Liang and others points out [9] that multidrug-resistant bacteria are a product of the unreasonable use of antibiotics. Some multidrug-resistant bacteria have been found in the pus of patients' ears, which poses a significant challenge to global healthcare management. On the other hand, the emergence of multidrug MRSA is one of the most common multidrug-resistant bacteria, with a separation rate of 22.8% in a study conducted in South Korea. A survey in the United States indicates that the incidence of community-acquired MRSA infections leading to chronic suppurative otitis media (CSOM) increased from 0.7% in 1998 to 11.4% in 2006. A study in India suggests that MRSA infections account for approximately 34.3% of the total *Staphylococcus aureus* in chronic suppurative otitis media (CSOM). Antibiotics can temporarily address OM and

reduce the number of ear-related pathogens, but they may disrupt the normal protective microbiota, allowing ear-related pathogens to recolonize and cause recurrent infections. Conservative treatment is the preferred choice for all cases of CSOM. Surgery is only used for patients who do not respond to drug treatment or who develop complications. Topical antibiotics, with or without steroids, and systemic antibiotics can be used to treat chronic suppurative otitis media (CSOM). Quinolones (ciprofloxacin, ofloxacin, levofloxacin) and aminoglycosides (gentamicin, neomycin, polymyxin B) are the most commonly used medications. The preference for local treatment is based on the following evidence: local treatment can be performed on an outpatient basis, is less expensive, and is generally considered to be more effective than systemic treatment [10]. Based on this, we need to find new ways to improve the efficacy for patients with otitis media in order to reduce the resistance caused by the clinical use of antibiotics.

3. Pathogenesis of Inflammatory Factor-Induced Infections

3.1. TLRs Impair Neutrophil Migration and Regulate Macrophage Inflammatory Responses

OM is a multifaceted disease. Bacterial infection is the dominant factor in most OM. Bacterial infection rapidly activates the host mucosal immune response, inducing leukocyte infiltration, mucosal hyperplasia, and exudation in the middle ear. The intrinsic mucosal immune system is characterized by epithelial and other mucosal cells with both anti-infective and barrier functions. The middle ear mucosa acts as a major barrier in OM pathology, as it is the first line of defense against bacteria [11]. One of the major advances in the recognition of microorganisms by the natural immune system is the recognition of Toll-like receptors (TLRs), which act as receptors for pathogen-associated molecular patterns (PAMPs), recognising them and triggering host defences, and are pattern-recognition receptors expressed by leukocytes, endothelial cells and parenchymal cells, which recognise pathogen-associated pattern molecules (pathogen-associated molecular pattern, PAMP) and endogenous damage associated molecular pattern (DAMP) [12] [13]. Ten TLR family members in humans and 11 in rodents allow cells of the innate immune system to respond with appropriate intracellular signals to the presence of all microbes. For example, Toll-like receptor 2 (TLR2) recognizes different toxins from Gram-positive bacteria, such as peptidoglycan, while TLR4 recognizes lipopolysaccharide (LPS) from Gram-negative bacteria. Signaling through TLRs is considered an important element of host defense during infection [14]. Recent data suggest that TLRs play a crucial role in the inflammatory response to bacteria in the ear and that these receptors are particularly important in the recovery of mice from OM. For example, TLR2 mutations delay bacterial clearance from the mouse middle ear and may lead to sepsis and death. TLR4 gene-deficient C3H/HeJ mice tend to develop spontaneous chronic otitis media. Deletion of Toll-like receptor 9 significantly prolongs the NTHi-induced inflammatory response in the middle ear and delays bacterial clearance [11] [15]. TLR4 is the most studied of the toll-like

receptor family. It is predominantly expressed in mononuclear phagocytes. TLR4 recognizes the bacterial cell wall product lipopolysaccharide (LPS) in the presence of CD14. LPS is a major contributor to the uncontrolled inflammatory response that leads to sepsis following Gram-negative bacterial infections and is often used as an experimental tool for elucidating the signaling events that are activated by Gram-negative bacteria. Negative regulators of Toll-like receptor 4 mediate the macrophage inflammatory response [16]. (Dong *et al.*, 2021: p. 1) [17] demonstrated that TLR4 is involved in regulating the formation of neutrophil extracellular traps (NETs) through the activation of autophagy and the generation of reactive oxygen radicals (ROS), and that TLRs promote the synthesis and release of cytokines, recruit immune cells, and trigger the cascade transduction of inflammatory signaling pathways. TLRs can promote the synthesis and release of cytokines, recruit immune cells, trigger the cascade of inflammatory signaling pathways, induce the inflammatory response of the middle ear mucosa, and also enhance the ability of the immune system to clear pathogenic microorganisms and activate acquired immunity. TLRs play an important role in the development of acute infection, chronic inflammation, and even neoplastic diseases, and in-depth study of TLRs can provide new ideas for the study of human anti-infective immunity, autoimmunity, immune tolerance, tumor mechanism, and vaccine adjuvant [18].

3.2. Role of NF-κB (p50/p65) in Bacterial Infections

NF-κB is a complex of cytoplasmic transcription factors, including RelA (p65), RelB, c-Rel, NF-κB1 (p50), and NF-κB2 (p52), that binds to specific binding sites on the promoters of target genes in the form of homo- or heterodimers. The activation of the NF-κB pathway leads to a wide range of inflammation-related gene expression, including cytokines, chemokines, and adhesion molecules. Therefore, controlling the activity of NF-κB is essential for maintaining the resting state and the alleviation of inflammatory responses; p65, as an important member of the NF-κB family, plays an important role in regulating the transcription of target genes. In the inactive state, p65 binds to IκB proteins in the cytoplasm and is not transcriptionally active. In the presence of inflammatory factors and other stimuli, IκB upstream kinase (IKK) is activated by phosphorylation, leading to IκB degradation. Many inflammatory mediators, including TNF-α, enter the nucleus through IκB α degradation and translocation of the p50-p65 dimer, and then p65 binds to specific DNA sequences to induce classical NF-κB activation, which binds to promoters of pro-inflammatory, anti-apoptotic, and proliferation-related genes [19]-[21]. It can function as an integrator of innate inflammatory signals. In normal epithelial cells, the major transcriptional activation subunit is the 65 kDa transactivating subunit RelA, a protein that is maintained in an inactivated state in the cytoplasm by binding to IκB proteins [22]. NF-κB is a pleiotropic regulator of many immune and inflammatory response processes, responding to injury and infection as well as promoting cellular proliferation and survival, which are

mediated through cell cycle regulation and growth factor stimulation mediation, and is an important factor involved in the transcriptional regulation of genes involved in immunity, inflammatory response, and cell differentiation [23] [24].

4. Innate Signaling in Otitis Media: Pathogenesis

4.1. TLR4 and Pathogenesis of Otitis Media

Among TLRs, TLR4 is a specific receptor for one of the molecular components of the Gram-negative outer membrane, lipopolysaccharide (LPS), and its truncated bodies, lipooligosaccharides (LOS) and lipid-like A, collectively known as endotoxins. TLR4 also recognizes endogenous molecules released by injured tissues and necrotic cells, known as damage-associated pattern molecules (DAMPs) molecules. The TLR4-mediated inflammatory response mediated by PAMPs and DAMPs is triggered and is involved in a variety of acute and chronic diseases. Thus, TLR4 is a key receptor for triggering pro-inflammatory responses at the convergence of non-infectious and infectious stimuli. TLR4 can exert either inflammatory or reparative effects. In general, inflammation has a protective function, and TLR4 can play an important role in this context, in particular by activating the induction of specific catabolic pathways that restore tissue integrity and function. However, when the TLR4 inflammatory response is not regulated, it can be detrimental to the organism [25]. Polymorphisms arising from the conversion of amino acids aspartic acid (Asp) and glycine (Asp299Gly) at position 299 of the Toll-like receptor 4 gene are of great importance. It has been shown [26] that the Asp299Gly variant of the TLR4 gene has an increased density of recurrent otitis media, and it is well known that *Streptococcus pneumoniae* (*Streptococcus pneumoniae*, SP) is the most common causative agent of acute middle ear infections. *Streptococcus pneumoniae* (*Streptococcus pneumoniae*, *S. pneumoniae*) produces large amounts of hydrogen peroxide in the infected environment. Hydrogen peroxide is an important oxygen radical mediator. Oxygen free radicals are especially caused by macrophages in the ligaments; thus, fatal damage occurs in this region. Macrophages in these ligaments secrete a variety of TLRs. TLR4 is a member of the TLR family of TLRs and plays an important role in responding to *Streptococcus pneumoniae* infections. Studies have confirmed that tissue damage and sclerosis occur within 9 hours during middle ear infections. Studies have shown that individuals carrying TLR4 variant genotypes are at increased risk of invasive disease during *Streptococcus pneumoniae* infection. Nuclear factor E2-related factor 2 (Nrf2) is involved in the regulation of oxidative damage mainly through the upregulation of antioxidant genes. In addition to this, Nrf2 regulates the inflammatory response during bacterial infection and is involved in the progression of LPS-induced AOM. Crosstalk between Nrf2 and the TLR pathway is present in the regulation of inflammation during the fight against infection. Nrf2 protects against acute lung injury and inflammation by modulating the TLR4 signaling pathway and regulates LPS-induced AOM by targeting TLR4 to inhibit inflammation and oxidative stress. Tuoheti *et al.* found [15] that the Nrf2/TTLR4

pathway may regulate the pathogenesis of CSOM, with higher expression of Nrf2 in mucosal tissues of chronic suppurative otitis media than in the chronic suppurative otitis media group and the non-chronic suppurative otitis media group, and a negative correlation between the mRNA levels of Nrf2 and TLR4 and the mucosal tissues of chronic suppurative otitis media. TLR activation results in the articulatory TLR activation, which leads to the recruitment and activation of protein molecules, including myeloid differentiation factor 88 (MyD88) and TIR structural domain-containing inducible IFN-β (TRIF). All TLRs except TLR3 can signal through the MydD88-dependent pathway to produce pro-inflammatory cytokines, such as tumor necrosis factor (TNF) and ILs, through nuclear factor-κB (NFκB) activation [27]. In a complex process, lipopolysaccharide-binding proteins transfer LPS to the TLR4 co-protein differentiation cluster 14 (CD14). In a second step, LPS is transferred to the next TLR4 accessory molecule, called myeloid differentiation protein-2 (MD-2). At least two TLR4/MD2/LPS complexes are required. They can form dimers, which initiate intracellular signaling pathways. The TLR4 signaling pathway consists of two distinct signaling pathways: the myeloid differentiation factor 88-dependent signaling pathway and the MyD88-independent signaling pathway. These mediators are key components of the innate immune response and are prepared to initiate the subsequent adaptive immune response through leukocyte chemotaxis and lymphocyte activation [28] [29]. TLR signaling is critical for the timely resolution of bacterial OM. Leichtle *et al.* found experimentally [28] that in mice lacking key TLR signaling molecules, expression of pro-inflammatory cytokines and macrophage activation for phagocytosis and killing of bacteria were severely disabled. This leads to failure of bacterial clearance and persistent OM. Defects in OM recovery are most prominent when certain key components of TLR signaling are absent, in particular TLR2, MyD88, and TNF. Si, Yu *et al.* suggested in their article that TLR2/TLR4-positive cells were predominantly found in the granulation tissue and were significantly more numerous than in normal external auditory canal skin, but fewer in the cholesteatoma capsule wall than in the normal external auditory canal skin. The results suggest that TLR2 and TLR4 are expressed in normal external auditory canal skin, chronic suppurative otitis media, and middle ear cholesteatoma, suggesting that the middle ear has an intrinsic immune system that is regulated by the involvement of TLR2 and TLR4, but the differential expression of the two also suggests that they play different roles in the pathogenesis of chronic suppurative otitis media and middle ear cholesteatoma.

4.2. Regulation of Nuclear Factor κB in Otitis Media

In the Milanovic *et al.* study [30], it was revealed that TNF and IL-1 (interleukin 1) induced significant structural changes in nuclear p65, detected by limited protein hydrolysis experiments and selective recognition of repetitively denatured p65 by conformation-specific antibodies. These induced structural changes were mainly mediated by post-translational p65 modifications. Individual NF-κB p65

phosphorylation induces structural changes in p65 that control subsequent ubiquitination and binding to transcriptional cofactors, thus contributing to the specificity of the NF-κB response. Post-translational modifications of NF-κB, including phosphorylation, acetylation, and methylation, have emerged as important regulatory mechanisms controlling the transcriptional outcome of this important transcription factor. These modifications act independently, sequentially, or in combination to regulate the multiple biological functions of NF-κB in cancer and inflammatory responses [31]. NF-κB function can be activated by bacterial products such as lipopolysaccharides (LPS), which underlie major global health burdens such as sepsis and endotoxemia. NF-κB activation in response to stimuli such as LPS is clearly characterized: prior to stimulation, inactivated NF-κB dimers are retained in cytoplasmic lysate by IκB proteins. Upon stimulation, the IκB kinase complex disrupts this retention, allowing nuclear translocation of NF-κB, which can induce target genes [32]. Data from Yanchun Feng *et al.* showed [21] that LRRC25 can be significantly upregulated upon activation of the NF-κB signaling pathway stimulated by LPS or TNF-α. Overexpression of LRRC25 inhibited LPS- and TNF-α-activated NF-κB signaling of LRRC25 in THP-1 cells significantly enhanced NF-κB activation and inflammatory factor production pathways in a dose-dependent manner. Knockdown of LRRC25 in THP-1 cells significantly enhanced NF-κB activation and inflammatory factor production. The LRR structural domain of LRRC25 was responsible for its inhibitory effect on the NF-κB signaling pathway. Specifically, the LRR structural domain of LRRC25 interacts with the RHD structural domain of p65/RelA and mediates the degradation of p65/RelA activation of NF-κB signaling stabilizes the LRRC25 protein, which in turn mediates the negative feedback regulation of p65/RelA by facilitating the interactions between p65/p50 and p62, which in turn promotes autophagic degradation. Xie and Gu *et al.* found that catalase lipooligosaccharide induced excessive middle ear inflammation through a cellular crosstalk mechanism during OM [33]. Lipooligosaccharide (LOS) selectively enhanced intercellular adhesion molecule 1 (ICAM-1 or CD54) on human monocytes by significantly increasing the intensity of surface expression and the percentage of ICAM-1 + cell ICAM-1 up Regulation by LOS on human monocytes requires surface CD14, TLR4, NF-κB p65, and c-Jun N-terminal kinase (JNK) activity. LOS also stimulates the production of pro-inflammatory cytokines by human monocytes in TLR4- and CD14-dependent pathways. The results of the study showed [1] that TLR2, 4 and NF-κB p65 (S276) mediate chronic inflammatory and immune responses in different types of chronic otitis media in children and adults. There was no difference in the expression of TLR2, 4 in otitis media (TTO) granulation tissues of the Eustachian tube tympanic cavity versus cholesteatoma (Ch) periportal stroma in adults. The percentage of TR2, 4-immunopositive cells was significantly lower in oedema and polyps of the patient's TTO than in the granulation tissue and peri-choroidal stroma of Ch. Another study showed that mRNA and protein expression levels of TLR2 were higher in the granulation tissue of chronic suppurative otitis media than in chronic

suppurative otitis media. In contrast to the differences in the percentage of cells exhibiting immunoreactivity for TLR2, 4 in different chronic otitis media tissues, nuclear immunoreactivity for NF-κB p65 (S276) was found to have a strong (more than 66% of immunoreactive cells) expression in all tissue samples. The strong nuclear immunoreactivity of NF-κB p65 (S276) among inflammatory cells in all tissue samples suggests that NF-κB p65 (S276) may mediate the inflammatory response in chronic otitis media.

5. New Treatments and Perspectives for Otitis Media

5.1. TLR-Targeted Therapy

TLR4 plays an important role not only in the inflammatory response but also in the post-inflammatory repair process. TLR4 signaling is activated by bacterial molecules and endogenous factors. TLR4 activators (agonists) and inhibitors (antagonists) are potential vaccine adjuvants and antitumor (agonists) and anti-inflammatory (antagonists) agents, respectively. Several new synthetic TLR4 modulators have been identified in the last 4 years; some of these compounds are chemically related to lipid A, the natural TLR4 ligand produced by bacteria. Other synthetic molecules, such as neoheptapeptides, Ugi compounds, chalcone, and morphine derivatives, have chemical structures that are not related to lipid A. Some natural compounds show TLR4 activity: saturated fatty acids and oxidized phospholipids have agonist effects [25]. Anti-LPS strategies that neutralize LPS (synthetic anti-LPS peptides and recombinant factor C) and TLR4/MyD88 antagonists include eritolan, a synthetic lipidoid A that binds to MD-2 and prevents LPS from activating TLR4, and therefore can be used to treat different inflammatory diseases [29]. LPS is a potent TRL4 agonist that induces strong inflammatory responses and has been shown to initiate the immune system and provide protection against infectious agents. However, it is not a therapeutic candidate due to its severe toxicity in humans. The vaccine adjuvant MPLA has immunomodulatory properties similar to those of LPS, showing protection against both Gram-negative and positive infections, and is well tolerated in humans. MPLA has 1/100th the toxicity of LPS, enhances innate immunity through expansion and mobilization of leukocytes to the site of infection, enhances phagocytosis and respiratory bursts, and provides antimicrobial resistance. However, MPLA is only approved for clinical use as a vaccine adjuvant and therefore cannot be used as a stand-alone antimicrobial agent. This creates a need for TLR4 agonists with similar immunomodulatory properties to MPLA that can be used as antimicrobial adjuvants for clinical use, such as PHAD and other clinically relevant TLR4 agonists. With the rising rate of antimicrobial resistance, TLR4 agonists may play an adjunctive role in limiting the time required for antibiotic therapy and potentially reducing the rate of infection [12]. Leichtle *et al.* [34] demonstrated that activation of the TLR2-NOD2-TNF signaling axis controls the inflammatory response of the middle ear through positive and negative modulators during the COM process. This complexity provides opportunities for individual differences to modulate mucosal responses

in the OM, leading to OM resistance or predisposition. It also provides multiple opportunities for pharmacological interventions to reduce pro-inflammatory signaling or enhance negative regulation.

5.2. NF-κB Signalling Pathway Therapeutic Targets

The NF-κB signaling pathway is mainly involved in carcinogenesis, immunity, and inflammation-related human diseases, and therefore a large number of natural and synthetic NF-κB inhibitors have been designed and discovered to inhibit the activation of the NF-κB signaling pathway. The search for biologically active inhibitory molecules capable of interfering with and/or blocking specific signaling pathways leading to NF-κB activation without multiple off-targets has become extremely challenging, and a number of NF-κB modulators and blocking strategies have been employed or are undergoing rigorous evaluation in laboratory studies and clinical settings. The basic mechanisms and strategies that may be included in the usual therapeutic NF-κB inhibitors are: 1) Blocking the original physiological stimulus signals that drive NF-κB activation. Rexatovir (TAK-242), a small-molecule inhibitor of Toll-like receptor 4 (TLR4) signaling, selectively binds to TLR4 and interferes with the interaction between TLR4 and its interface molecules, thereby blocking downstream NF-κB activation. 2) Targets the phosphorylation pathway associated with NF-κB activation; NF-κB phosphorylation controls transcription in a gene-specific manner; C25-140 can target TRAF6 E3 ligase activity and inhibit downstream NF-κB phosphorylation. 3) Regulation or activation of the IκB complex or other NF-κB subunits. BAY11-7082, an inhibitor of IκB*α* phosphorylation and NF-κB, acts through TNF-*α*-induced IκB-*α* phosphorylation, resulting in reduced nuclear factor-κB and adhesion. κB-*α* phosphorylation induced by TNF-*α*, leading to a decrease in the expression of nuclear factor-κB and adhesion molecules. 4) Block NF-κB translocation, DNA sequence recognition, and binding and/or modification of NF-κB that affects its activity or targeting specificity. JSH-23 inhibits nuclear translocation of NF-κB p65 without affecting IκB*α* degradation. Long-term systemic intake of low-dose NF-κB inhibitors, including dietary administration of lignans, diterpenes and sesquiterpenes, saponins, polysaccharides, polybiofibres, and other natural products with generally beneficial effects, acts through chronic inhibition of inflammatory signaling induced by NF-κB activity, oncogenic effects [35]. The typical NF-κB signaling pathway is a mediator of cellular inflammatory responses and a target for the development of therapeutic agents for a wide range of human diseases. While a large number of compounds are known to inhibit upstream proteins in the activation pathway, the most downstream protein in the pathway—the p50/p65 transcription factor heterodimer—has been refractory to small-molecule inhibitors. Given the role of many upstream proteins in multiple biochemical pathways, targeting the p50/p65 heterodimer offers the opportunity to enhance ontology specificity. To this end, the p65 protein has two non-disulfide cysteines (Cys38 and Cys120) at its DNA-binding interface that are suitable for covalent molecular targeting.

The natural product helenalin, a sesquiterpene lactone, has previously been shown to target Cys38 on p65 and impair its DNA-binding capacity [36]. However, it should be taken into account that many of the new treatment options, although very interesting, have not yet been adequately tested in humans in clinical trials and therefore, if effective, they will be available for clinical use for many years. Currently, available guidelines and recommendations for OM approaches remain essential while waiting for new potentially effective preventive and therapeutic measures. Although largely perfect, they still represent the best solution for ensuring acceptable OM methods, improving their diagnosis, reducing antibiotic abuse and misuse, and avoiding unnecessary surgery.

Conflicts of Interest

There are no interests or disputes in this article.

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