applied microbiology

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Review

Recurrent Acute Otitis Media Environmental Risk Factors: A Literature Review from the Microbiota Point of View

Francesco Folino [1,*], Pietro Bosi [1], Sara Torretta [2,3], Michele Gaffuri [2,3] and Paola Marchisio [4,5]

[1] Università degli Studi di Milano, 20122 Milan, Italy
[2] Department of Otolaryngology and Head and Neck Surgery, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, 20122 Milan, Italy
[3] Department of Clinical Sciences and Community Health, Università degli Studi di Milano, 20122 Milan, Italy
[4] Pediatria Alta Intensità di Cura, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, 20122 Milan, Italy
[5] Department of Pathophysiology and Transplantation, Università degli Studi di Milano, 20122 Milan, Italy
* Correspondence: francesco.folino@unimi.it

Abstract: Acute otitis media (AOM) constitutes a multifactorial disease, as several host and environmental factors contribute to its occurrence. Prevention of AOM represents one of the most important goals in pediatrics, both in developing countries, in which complications, mortality, and deafness remain possible consequences of the disease, compared to in developed countries, in which this condition has an important burden in terms of medical, social, and economical implications. The strategies for AOM prevention are based on reducing the burden of risk factors, through the application of behavioral, environmental, and therapeutic interventions. The introduction of culture-independent techniques has allowed high-throughput investigation of entire bacterial communities, providing novel insights into the pathogenesis of middle ear diseases through the identification of potential protective bacteria. The upper respiratory tract (URT) is a pivotal region in AOM pathogenesis, as it could act as a source of pathogens than of protective microorganisms for the middle ear (ME). Due to its direct connection with the external ambient, the URT is particularly exposed to the influence of environmental agents. The aim of this review was to evaluate AOM environmental risk factors and their impact on URT microbial communities, and to investigate AOM pathogenesis from the microbiota perspective.

Keywords: recurrent acute otitis media; environmental risk factors; microbiota; childhood

Citation: Folino, F.; Bosi, P.; Torretta, S.; Gaffuri, M.; Marchisio, P. Recurrent Acute Otitis Media Environmental Risk Factors: A Literature Review from the Microbiota Point of View. *Appl. Microbiol.* **2022**, *2*, 594–613. https://doi.org/10.3390/applmicrobiol2030046

Academic Editor: Bong-Soo Kim

Received: 30 June 2022
Accepted: 12 August 2022
Published: 18 August 2022

1. Introduction

Acute otitis media (AOM) is the most common bacterial infection and the leading cause of antibiotic prescription in childhood [1,2]. It is defined as the presence of fluid in the middle ear (ME) with signs/symptoms of acute infection [3]. Recurrent acute otitis media (RAOM), defined as the occurrence of three AOM episodes in 6 months or four AOM episodes in 12 months [4,5], implicates a great effort in assistance considering the burden of medical visits, drug prescription, and the loss of working days for parents.

The etiological agents of AOM, defined as otopathogens, comprehend *Streptococcus pneumoniae*, non-typeable *Haemophilus influenzae*, *Moraxella catarrhalis*, and *Streptococcus pyogenes* [6]. The upper respiratory tract (URT) represents a pivotal region in the pathogenesis of the disease. These microorganisms usually colonize the URT during infancy and constitute part of the normal nasopharyngeal flora; according to the Pathogen Reservoir Hypothesis (PRH), the URT and the adenoid pad serve as a source of pathogens that become more virulent whereas favorable circumstances impair the homeostasis established among bacteria, viruses and the host immune system [7–10], further spreading to the middle ear and respiratory system [11–14].

In recent years, the introduction of next-generation sequencing (NGS) techniques has allowed high-throughput analysis of entire bacterial communities, with a subsequent modification of our approach to investigation and management of infectious diseases, now studied under an ecological perspective: health and disease are determined by a complex balance among pathogens, host immune response and resident microbiota, not by the sole presence or absence of a pathogen [15].

The human microbiota comprehends communities of commensal, symbiotic and pathogenic microorganisms that colonize different body sites, such as the gastrointestinal tract, respiratory system, oral cavity, skin, and female reproductive system [16]. These microorganisms and their metabolites fulfill a significant role in defense against pathogens [17], immune response, and inflammation [18].

In the last decade, most of the studies on microbiota in the URT have focused on the identification of potential keystone species, that is "species that are typically not highly abundant but are disproportionately important in maintaining the organization and structure of an entire community" [19]; in particular, in the URT, *Dolosigranulum* spp. and *Corynebacterium* spp. have been identified as potential keystone species and have been related to respiratory health and exclusion of otopathogens [20–23].

The microbial communities and their composition are extremely dynamic and change under the effect of several external agents, especially in infancy and early childhood [24,25]. An impairment in the composition and consequently in the homeostatic function of the microbiota mediated by these environmental factors leads to a condition, defined as dysbiosis, which can result in immediate or long-term effects on the health status [26].

AOM is a multifactorial disease, as diverse agents contribute to its occurrence [27]. Risk factors can be distinguished into two categories: host and environmental. The first group includes age, sex, ethnicity, family history of AOM and genetic predisposition, craniofacial anomalies, atopy, immunodeficiency, adenoid hypertrophy, gastroesophageal reflux [27]; environmental factors include day-care attendance, passive smoking, older siblings, use of pacifier, no breastfeeding [27], pollution [28], season [29], delivery route [30]. Prevention of AOM represents one of the most important goals in pediatrics, both in developing countries, in which complications, mortality, and deafness remain possible consequences of the disease, than in developed ones, in which this condition has an important burden in terms of medical, social and economical implications [31,32]. The strategies put into practice for AOM prevention are mostly based on reducing the burden of risk factors while improving protective ones, through the application of behavioral, environmental, and therapeutic interventions.

The URT and its microbiota are especially exposed to environmental factors, as this region represents the interface between the external environment and the respiratory system and is interconnected with the middle ear, lower airways, and gastrointestinal tract. The most important AOM environmental risk factors likely intervene in AOM pathogenesis through the induction of major modifications and shaping of the URT microbiota. A deepened understanding of the most important characteristics of the URT microbiota and of the influence of the external conditions on its balance could provide major insights into producing probiotic therapies and improving our preventive programs.

The aim of this review is to evaluate AOM environmental risk factors and their role in the pathogenesis of the disease from the microbiota point of view through the analysis of evidence available in microbiome research on this topic.

2. Materials and Methods

The research was conducted on the PubMed database, including all evidence available until December 2021. MeSH terms such as "otitis media", "microbiota", "child", "child, preschool" and "infant" were used. More articles were included combining the keywords "microbiota" and "microbiome" with terms such as "acute otitis media", "risk factors", and "nasopharyngeal".

After the exclusion of duplicates, 103 potentially relevant studies were identified through this search strategy. After title-abstract analysis, 71 studies were excluded as nonpertinent, according to the following criteria: studies not coherent with the aim of this review; site of investigation different than URT; studies not evaluating risk factors for OM. Of the remaining 32 articles, 16 were further excluded as review/editorial articles, adult population, and studies conducted with culture-dependent techniques. A total of 16 articles were therefore considered for this review (see Figure 1 for more details on methods).

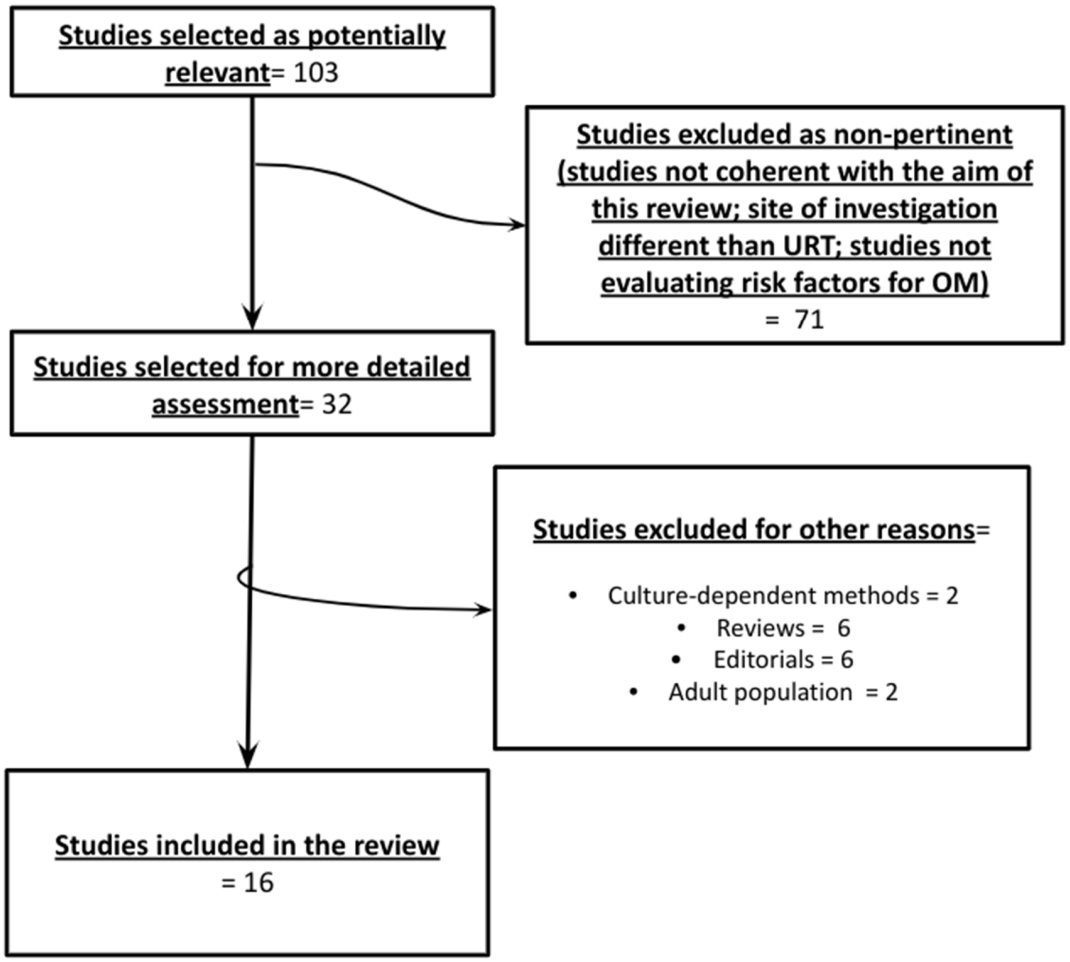

Figure 1. Search strategy conducted for this review.

3. Environmental Risk Factors of AOM and URT Microbiota

3.1. Breastfeeding

Breastfeeding is a well-known protective factor against infection [33,34] and is tightly connected to the prevention of AOM recurrences [35,36]. In particular, exclusive breastfeeding and a longer duration of this practice are associated with a lower risk of AOM [37]; in a meta-analysis by Bowatte et al., exclusive breastfeeding during the first 6 months was associated with a 43% reduction in ever having AOM in the first 2 years of life [38].

This protective effect is surely related to the high amount of antibacterial substances in maternal milk [39]. Nevertheless, in the last decade evidence has enhanced the role of breastfeeding and its major impact on the development of healthy microbial communities.

Biesbroek et al. analyzed nasopharyngeal microbiota in 101 healthy breastfed infants and 101 formula-fed infants at the ages of 6 weeks and 6 months: data showed a bacterial community enriched in common commensals, in particular *Dolosigranulum* and *Corynebacterium*, in those who were breastfed at 6 weeks of age; moreover, *Dolosigranulum* abundance was related to a lower number of respiratory tract infections in the following months. At 6 months, no clear differences were observed in microbial profiles: the authors

stated that the introduction of solid foods could reduce the dissimilarities in microbiota composition in the two groups. However, the early microbiota composition, especially an enrichment in *Dolosigranulum*, and its relation to breastfeeding, seemed to have an impact on respiratory health in later stages of life [40]. Another study by the same group confirmed the association between *Corynebacterium/Dolosigranulum* and *Moraxella* dominated profiles in early life to breastfeeding; moreover, these profiles appeared more stable over time and were associated with a reduced number of respiratory infections [41]. Similar results were provided by Bosch et al., in a study evaluating the potential environmental drivers of respiratory microbiota maturation. The authors identified features of nasopharyngeal microbiota in the first month of life that related to a higher number of respiratory infections: in particular, profiles characterized by a prolonged reduction of *Dolosigranulum* and *Corynebacterium* were associated with a higher number of infectious recurrencies. Vaginal delivery and breastfeeding were two important determinants of this composition, as a major abundance of these genera was observed in breastfed infants. However, analogously to previous studies, these dissimilarities were transient and disappeared around 6 months of age [42].

The relation between breastfeeding and *Dolosigranulum* enriched communities was further confirmed in another investigation evaluating URT microbiota in children with invasive pneumococcal disease (IPD); a higher abundance of *Dolosigranulum* was not only related to breastfeeding, but also had a negative correlation with *S. pneumoniae* abundance [43].

Concerning *Corynebacterium* spp., interesting data were provided in a more recent study describing the nasopharyngeal microbial communities and environmental factors that potentially shape their composition in the first year of life. Even in this case, higher abundances of *Corynebacterium* spp. were associated with a lower risk of *S. pneumoniae* colonization; feeding practices were one of the major drivers shaping the composition of microbial communities. In particular, breastfeeding was associated with an enrichment in *Corynebacterium* [44]. URT microbiota in IPD was also evaluated in a study by Henares et al., in which different respiratory health statuses were evaluated comparing three groups of children: 27 patients with IPD, 48 with upper respiratory tract infection (URTI), and 65 asymptomatic controls. Interestingly, microbiota composition varied with disease severity, as the IPD group was characterized by a higher representation of *S. pneumoniae* and a reduced abundance of *Dolosigranulum*. Among the environmental factors considered, a longer duration of breastfeeding seemed to influence the microbiota structure, even if the authors were not able to evidence a significant enrichment in beneficial bacteria in children breastfed for at least 6 months [45].

The potential mechanisms through which breast milk can shape the URT microbiota have been evaluated by Binia et al. in a complex and interesting study characterizing nasopharyngeal microbiome by shotgun metagenomics: mother-infant dyads in which children were breastfed for at least 4 months were selected, for a total of 240 infants included; mothers were further classified according to their secretory status by sequencing of FUT2 and FUT3 genes, which shape fucosylated human milk oligosaccharides (HMOs) in breast milk. HMOs are retained to be one of the key components of human milk in determining its protective action against infections [46,47]. A positive maternal secretor status was associated with a lower risk of acute respiratory infections in the first 6 months of life, suggesting a protective role of 1, 2-fucosylated HMOs in early infancy. However, the authors could not find evidence that this protective effect of HMOs is conveyed by the modulation of microbiota and could not relate its composition at 2 and 4 months to a subsequent acute respiratory infection (ARI) risk. The authors thus concluded that breastfeeding and HMOs could act through an immunomodulatory effect, rather than through a direct impact on microbiota composition [48]. Therefore, the higher abundance of *Corynebacterium* and *Dolosigranulum* does not appear to be related to the presence of HMOs in human milk. To date, the enrichment with these two genera in breastfed infants is more likely related to the composition of the human milk itself rather than to its metabolic

effects. For instance, *Corynebacterium* spp. have been frequently isolated from human milk [49–51], while clear data are lacking concerning *Dolosigranulum*, even if its presence has been also described in human mature milk [52]. Moreover, *Corynebacterium* spp. are known common colonizers of the human skin [53], so it is likely that its enrichment in breastfed infants is supported by the close contact of the infants' respiratory tract with the maternal skin during breastfeeding. Notably, it is also plausible that the enrichment in *Dolosigranulum* in breastfed infants is mediated indirectly through *Corynebacterium* spp. It is indeed well-known that these two microorganisms are often found to be co-occurrent in the URT, and in vitro assays have shown that certain nasal *Corynebacterium* spp. can enhance the growth of *Dolosigranulum* [54].

3.2. Delivery Route

Vaginal delivery represents another significant protective factor against infections. Children born by caesarian section (CS) experience a higher number of subsequent respiratory recurrences in comparison with children born by vaginal delivery [55,56]. AOM is clearly included among this group of infections: evidence shows a highly significant increased risk for >3 episodes of OM in children born by CS [30]. Moreover, a more recent retrospective study on a high sample size conducted in Canada and including 36.318 children showed that CS delivery is associated with a slightly higher risk of OM and a higher number of OM episodes [57].

One of the potential mechanisms explaining the association between CS and infection is related to the alterations in innate and adaptative immunity described in children born by CS [58]. However, one of the most interesting aspects concerns the different microbial colonization patterns in different body sites in relation to the delivery route. At birth, the majority of the microorganisms encountered by the newborn are provided by the mother's vaginal environment through the passage in the birth canal [56]. This initial colonization step, irretrievably connected to the delivery route, is crucial in defining the subsequent ecosystems and their development trajectories, therefore influencing the health status in the following years [59]. Concerning nasopharyngeal microbiota, the first bacterial communities after birth were initially described by Dominguez-Bello et al., in a study in which swabs were collected from different body sites from healthy neonates born vaginally or by CS immediately after birth: in the first phases of life, undifferentiated microbial communities were identified; interestingly, these communities resembled vaginal microbiota in children vaginally delivered, while they were more similar to maternal skin microbiota in those who were born by CS. For instance, microbiota in vaginally delivered children was enriched in *Lactobacillus*, *Prevotella*, *Atopobium*, or *Sneathia* spp., while children born by CS had communities enriched in skin commensals, as *Staphylococcus* spp. [60].

A subsequent prospective cohort study evaluated the nasopharyngeal microbiome in 102 children through their first 6 months of life, providing interesting insights on the developmental patterns of URT microbiota in relation to the delivery route. Data show that a nasopharyngeal niche differentiation occurs at one week of age, characterized by a higher abundance of *S. aureus* in almost all children; a subsequent reduction of *S. aureus* is replaced by *Corynebacterium* spp. and *Dolosigranulum* spp., followed by *Moraxella catarrhalis/nonliquefaciens*, *S. pneumoniae*, and *H. influenzae* in the first six months. Mode of delivery had a major influence on this process, as children born by vaginal delivery used to switch to *Moraxella* and *Corynebacterium/Dolosigranulum* dominated profiles at an earlier age compared to those born by CS; on the other hand, CS born children were longer colonized by *S. aureus* enriched communities. Moreover, not only vaginally delivered children were more likely to switch to *Corynebacterium/Dolosigranulum* dominated profiles, but communities of infants born by CS had a lower abundance of these potential protective bacteria [59].

The mechanisms underlying the correlation between delivery route and URT microbiota composition are not clearly defined. *Dolosigranulum pigrum* is included in the group of Lactic Acid Bacteria (LAB), which also encompasses *Lactobacillus* spp.; these microorgan-

isms are fundamental members of the vaginal microbiota, and especially the production of lactic acid has been associated with a healthy genital environment [61]. However, a recent study evaluated the habitat range of *Dolosigranulum* through the analysis of 8184 samples from different body sites: interestingly, it was not detected in vaginal samples [62]. As hypothesized by Bosch et al., the impact of delivery mode on URT microbiota is probably more related to indirect mechanisms rather than a direct effect of the maternal vaginal environment [59].

Moreover, the dissimilarities in microbial communities related to delivery mode appear to be transient until six weeks of age, thus the body site itself could have a major impact on the microbiota in the postnatal period [63].

3.3. Smoking

Smoking, whether active or passive, is a major risk factor for respiratory disease, as it induces ciliostasis, goblet cell hyperplasia, and mucus hypersecretion, which could cause accumulation of mucus and bacteria in the URT and subsequently in the middle ear [64]. Moreover, it has been shown that passive smoking favors nasopharyngeal colonization by otopathogens [65].

In our research, we did not find any study concerning nasopharyngeal microbiota and smoking influence on pediatric age. Moreover, most of the studies on this topic have focused on the oropharyngeal/gengival environment and have investigated the potential relation to periodontal disease. Some preliminary data have been provided by Charlson et al., who compared nasopharyngeal microbial communities between 29 smoking and 33 nonsmoking adults, showing a higher abundance of *Haemophilus* spp. and other gram-positive anaerobic lineages in smokers [66].

It is well-known that passive smoking has a major negative impact on the nasal mucosa, inducing loss of cilia, hyperplasia of goblet cells, vascular congestion [67], and favoring biofilm formation [68]. On this basis, it is reasonable to speculate that smoking could have an impact also on microbial communities, as a consequence of the damaged nasal mucosa.

However, further studies with more consistent data are surely needed to deepen our knowledge on this topic and to better define the negative impact of smoking on respiratory health from the microbiota perspective.

3.4. Daycare Attendance

Daycare attendance constitutes a strong risk factor for AOM, most likely due to the increased exposure to the main colonizing otopathogens and to respiratory viral agents [69].

In particular, an early introduction to community life is strongly associated with an incremental risk of AOM: in a prospective study conducted in The Netherlands, children who started daycare at 6–12 months of age suffered from a higher number of AOM episodes, received more antibiotic prescriptions and underwent more medical visits [70], while another observational study on 1056 children showed an increased risk for AOM by 22% every month of age less the baby started daycare in the first year of life [71]. On the other hand, as expected, a 1.5% risk reduction in having 3 or more episodes of AOM for each month not attending daycare has been described [30].

We did not find any study with a specific design on this topic in relation to OM and URT microbiota. Some data are provided by studies in which authors have investigated nasopharyngeal microbiota in children taking into account several risk and epidemiological factors, without a precise focus on daycare attendance.

For instance, in a landmark study by Bosch et al., the authors evaluated the most important environmental drivers in relation to URT microbiota development, describing how children undergoing a higher number of infectious recurrences had an aberrant microbiota development, defined by a prolonged reduction of *Corynebacterium* and *Dolosigranulum* and early enrichment of *Moraxella*. Among others, daycare attendance was associated with this aberrant trajectory, which related to worse clinical outcomes [42].

Xu et al. investigated nasopharyngeal (NP) microbiota by comparing stringently-defined otitis prone children (sOP) versus AOM-free children at 6 and 12 months of age, showing a different microbial profile and a reduced alpha diversity in the sOP group at 6 months. In particular, genera such as *Bacillus*, *Prevotella*, *Gemella*, *Veillonella*, and *Actinomyces* were less abundant in sOP samples. The two most important factors associated with these differences were *S. pneumoniae* colonization and daycare attendance, indicating that the latter might have a significant impact on the whole community composition and contribute to dysbiosis at this age [72].

Close contact with other children and continuous exposure to infectious agents, both viral and bacterial, likely impairs the normal composition of microbial communities, especially in the URT. Mounting evidence shows indeed that several viral infections that are common in childhood can impair the stability of the respiratory microbiota, as described in relation to rhinovirus [73,74], influenza [75], and respiratory syncytial virus [73].

In general, viral upper respiratory tract infections are related to a higher colonization rate by otopathogens, especially in symptomatic cases, as shown by studies conducted both with standard PCR than with NGS techniques [76,77]. On the other hand, an enrichment in otopathogens has been associated with a higher recurrence of upper respiratory tract infections [76].

Daycare attendance, through a repeating exposure to viral agents, could be a starting point for a vicious cycle of recurrent viral disease and subsequent AOM episodes. However, our knowledge of viral-bacterial interactions in the URT is not fully elucidated, mainly because of the intrinsic limit of the most commonly used high throughput techniques applied in microbiome studies, which prevent a comprehensive evaluation of the whole virome and bacteriome at the same time.

3.5. Season

The incidence of AOM is higher in autumn and winter and is lower during spring and summer. Cold seasons are indeed characterized by a higher incidence of upper respiratory tract infections, which often precede an AOM episode [78]. In particular, in a recent Korean study, December was identified as the most common month for AOM occurrence, while June through September had low AOM occurrence and July had the least number of AOM cases each year [56].

The composition of microbial communities is influenced by season at sample collection [79]. In one of the first studies evaluating URT microbiota in children, Bogaert et al. analyzed samples from 96 healthy children and compared microbiota composition in those collected in winter/fall versus those collected in spring, reporting for the first time differences in NP microbiota according to seasonality with an NGS technique; in particular, samples obtained in fall/winter had a higher abundance of *Proteobacteria*, *Fusobacteria* and *Cyanobacteria*, while samples collected in spring were significantly enriched in *Bacteroidetes* [80]. Moreover, in a previously cited investigation evaluating the role of HMOs in human milk on the URT microbiome through shotgun metagenomics, season at sampling was the most relevant environmental factor shaping the microbial communities [48].

Winter season has been associated with a reduced relative abundance of the potential protective genera *Corynebacterium* and with a higher abundance of *S. pneumoniae* in the same period of the year [44]. Longitudinal data have been provided by Mika et al., who analyzed 872 nasal swabs from 47 children during the first year of life: at the family level, *Corynebacteriaceae*, which include potential protective bacteria, were more abundant in summer months; on the other hand, *Pasteurellaceae*, which among others comprehend the *Haemophilus* spp., were more abundant during winter, showing an outgrowth of potential otopathogens during this season [81]. Not only do microbial communities seem to be influenced by season fluctuations, but also season at birth has an impact on nasopharyngeal microbiota; as shown by Schoos et al., early bacterial colonization at 1 month of age was influenced by the season of birth, as bacterial richness was higher in infants born in the summer [82].

Seasonality is associated with respiratory diseases, which usually occur more often in winter than during summer [83]. Taken together, data show that the microbial communities in the URT are influenced by seasonality, providing a potential additional explanation for the correlation between a certain period of the year and respiratory recurrencies. Several hypotheses can be considered to explain this correlation: first of all, as previously discussed, viral agents can impair the homeostasis in microbial communities, and the seasonality of circulation of respiratory viruses is widely recognized [84]; moreover, seasonal variations in immune system functions have been described, contributing to the incidence of respiratory infection and, in our opinion, potentially influencing the composition of microbial communities [83]; lastly, the composition of URT microbiota has been also correlated to levels of circulating vitamin D, whose production is enhanced during summer [85].

3.6. Presence of Siblings

The presence of siblings nearly doubles the risk of presenting an episode of AOM and enhances the risk of RAOM in the first two years of life [69,86]. However, available studies also agree that it is still hard to correctly value the weight of the genetic influence between siblings in comparison to the sharing of the same familiar environment [87].

The higher incidence of respiratory infections in children with older siblings is related not only to a higher risk of transmission of viral agents, as the possibility of bacterial pathogens transmission, including *S. pneumoniae*, from one sibling to the other one has been demonstrated [88]. It is therefore plausible that the presence of siblings could impact the whole community composition in the URT.

In a cross-sectional study of 105 healthy infants aged <1 year, infants with siblings were colonized by profiles enriched in *Moraxella* and lacking in *Corynebacterium/Dolosigranulum*, while only children had an opposite pattern [89]. A lower abundance of *Corynebacterium* in the URT of children with siblings was later confirmed in a longitudinal study evaluating determinants of nasal microbiota development in the first 18 months of life [90].

Concerning otopathogens, a study conducted by Bosch et al. showed an association between the presence of siblings and increased abundance of *Pasturellaceae*, which includes *Haemophilus* spp. [42].

3.7. Air Pollution

The association between air pollution and OM has been demonstrated in studies conducted both in vivo and in vitro. Pollution facilitates the development of OM by promoting apoptosis, expression of inflammatory cytokines (TNF-α and COX-2), and expression of mucin genes in vitro [91], while the injection of pollution particles into the ME of animals increases the thickness of the ME mucosa, promotes infiltration of inflammatory cells and inhibits the expression of epithelial sodium channels, whose purpose is to maintain the ME free of fluids [92]. In particular, an increase in the concentration of PM 2.5 appears to better correlate with the development of OM compared with PM 10, especially in children under 2 years of age [93]. On the other hand, a systematic review conducted by Bowatte et al. included 24 observational studies and concluded that the evidence of a causal relationship between pollutants and otitis media is still limited [94].

Evidence on the impact of pollution on the URT microbiota in the pediatric age is lacking. A recent longitudinal study evaluated the potential impact of greenness and air pollution on the nasal microbiota of 47 healthy infants in the first year of life, showing that increased NO_2 levels were related to a lower abundance of *Corynebacterium* and that low-to-moderate exposure levels could impact the URT microbiota composition in infants; on the other hand, no correlation was identified between PM 2.5 and abundance of *Corynebacterium* [95]. Potential mechanisms through which pollution could induce dysbiosis include modifications of the airway physiological environment and epithelial damage [96].

An overview of the studies discussed in this review is available in Table 1.

Table 1. Overview of investigations on microbiota and recurrent acute otitis media environmental risk factors discussed in this review.

Title (Year of Publication) [Ref]	Study Design	*N.* of Subjects	Age	Environmental Factor	Main Findings
The Impact of Breastfeeding on Nasopharyngeal Microbial Communities in Infants (2014) [40]	Comparison of NP microbiota between infants that had received exclusive breastfeeding and children that had received exclusive formula feeding	Exclusive breastfeeding (n = 101) Formula feeding (n = 101)	<6 m (samples collected at 6 weeks and 6 months of age in all subjects)	Breastfeeding	At 6 weeks of age: higher abundance of *Dolosigranulum* and *Corynebacterium* and decreased abundance of *Staphylococcus* and *Prevotella* in breastfed infants Predominance of *Corynebacterium* and *Dolosigranulum* was observed in 44.6% of the breastfed infants compared with 18.8% formula-fed infants; *Dolosigranulum* abundance was inversely associated with incidence of mild RTIs
Early Respiratory Microbiota Composition Determines Bacterial Succession Patterns and Respiratory Health in Children (2014) [41]	Longitudinal URT microbiota analysis in healthy children in the first 2 years of life	60 healthy children	<2 years (samples collected at 1.5, 6, 12, and 24 months of age)	Breastfeeding	Stable developmental patterns were characterized by early presence and higher abundance of *Moraxella* and *Corynebacterium/Dolosigranulum* *Moraxella* and *Corynebacterium/Dolosigranulum* dominated profiles were associated with breastfeeding and with lower rates of RTIs
Maturation of the Infant Respiratory Microbiota, Environmental Drivers, and Health Consequences A Prospective Cohort Study (2017) [42]	Longitudinal URT microbiota analysis in healthy children during the first year of life	112 infants (1121 samples)	<1 year (samples collected 2 h after birth, at 24 h, at 7 and 14 days, at 1, 2, 3, 4, 6, 9, and 12 months of age)	Breastfeeding Delivery route Daycare attendance	A higher incidence of RTIs in the first year of life was associated with an altered microbiota development from the first month of life on, consisting in a prolonged reduction of *Corynebacterium* and *Dolosigranulum*, early enrichment of *Moraxella*, later enrichment of *Neisseria* and *Prevotella* spp. Independent drivers of these aberrant alterations were delivery route, feeding practices, crowding, recent antibiotic use
Nasopharyngeal Microbiota in Children With Invasive Pneumococcal Disease: Identification of Bacteria With Potential Disease-Promoting and Protective Effects (2019) [43]	Comparison of URT microbiota in children with IPD and healthy controls	56 children (28 with IPD and 28 healthy children)	IPD group: 20.8–60.2 months (median 43 m) Control group: 31.6–58.9 (median 42.6 m)	Breastfeeding	*Dolosigranulum* dominated profiles appeared to be more resistant to pneumococcal infection occurrence and severity A higher proportion of *Dolosigranulum* dominated profiles was identified in healthy controls that were breastfed A significant negative correlation was observed between *Dolosigranulum* vs. *Streptococcus* (p = 0.029)

Table 1. *Cont.*

Title (Year of Publication) [Ref]	Study Design	*N.* of Subjects	Age	Environmental Factor	Main Findings
Non-diphtheriae Corynebacterium species are associated with decreased risk of pneumococcal colonization during infancy (2021) [44]	Longitudinal URT microbiota analysis in mother-infants dyads	179 mother–infant dyads (1368 infant and 172 maternal samples)	<1 year (NP swabs collected monthly between 0–6 months of age and bimonthly between 6–12 months)	Breastfeeding Season	Strong negative association between the relative abundance of *Corynebacterium* and *S. pneumoniae* colonization rate Breastfeeding was associated with an increase in *Corynebacterium* relative abundance Antibiotic exposures and the winter season related to a decline in the relative abundance of *Corynebacterium*
Differential nasopharyngeal microbiota composition in children according to respiratory health status (2021) [45]	Prospective case–control study. NP microbiota analysis in three groups of children: cases with IPD, symptomatic controls with mild viral URTI, and health controls	140 (IPD = 27; URTI = 48; healthy = 65).	IPD: 19.0–49.5 m (median 33 m) URTI: 14.7–45.0 m (median 24.5 m) Healthy controls: 19.0–43.0 m (median 31 m)	Breastfeeding	IPD group was characterized by a higher representation of *S. pneumoniae* and a reduced abundance of *Dolosigranulum* and *Moraxella lincolnii* A longer duration of breastfeeding seemed to influence the microbiota structure; however, the authors did not evidence a significant enrichment in beneficial bacteria in children breastfed for at least 6 months
The Influence of FUT2 and FUT3 Polymorphisms and Nasopharyngeal Microbiome on Respiratory Infections in Breastfed Bangladeshi Infants from the Microbiota and Health Study (2021) [48]	Longitudinal URT microbiota analysis through shotgun metagenomics in a cohort of breastfed infants and the potential influence of fucosylated human milk oligosaccharides (HMOs) on its composition and ARI incidence in the first two years of life	240 children (422 total samples)	<2 y	Breastfeeding Season	Maternal secretor status was associated with reduced ARI incidence from birth to 6 months HMOs could act through an immunomodulatory effect, rather than through an impact on microbiota composition Season at sampling was the most relevant environmental factor shaping the microbial communities
Development of Upper Respiratory Tract Microbiota in Infancy is Affected by Mode of Delivery (2016) [59]	Longitudinal NP microbiota analysis in the first 6 months of life; comparison between URT microbiota in vaginally delivered children with those born by CS	102 (Vaginal delivery = 62; CS = 40) 761 total samples	<6 m	Delivery route	Niche differentiation occurs in the first week of life, initially with *Staphylococcus aureus* predominance, followed by differentiation towards *Corynebacterium pseudodiphteriticum/propinquum*, *Dolosigranulum pigrum*, *Moraxella catarrhalis/nonliquefaciens*, *Streptococcus pneumoniae*, and/or *Haemophilus influenzae* dominated communities Delay in microbiota development and reduced abundance of *Corynebacterium* and *Dolosigranulum* in infants born by CS

Table 1. *Cont.*

Title (Year of Publication) [Ref]	Study Design	*N.* of Subjects	Age	Environmental Factor	Main Findings
Delivery mode shapes the acquisition and structure of the initial microbiota across multiple body habitats in newborns (2010) [60]	Characterization of bacterial communities from mothers and newborn babies in different body sites (mothers' skin, oral mucosa, and vagina; neonates' skin, oral mucosa, URT, and meconium)	10 (4 born vaginally, 6 born by CS)	Neonates (samples collected in the first hours of life)	Delivery route	Vaginally delivered infants acquire bacterial communities similar to their mother's vaginal microbiota (*Lactobacillus*, *Prevotella*, *Sneathia* spp.) CS delivered infants harbored bacterial communities similar to those found on the skin surface (*Staphylococcus*, *Corynebacterium*, and *Propionibacterium spp*.)
Nasopharyngeal microbiome analyses in otitis-prone and otitis-free children (2021) [72]	Comparison of URT microbiota in sOP and otitis-free children at 6 and 12 months of age	28 sOP children 68 AOM-free children (157 total samples)	<1 y (samples collected at 6 and 12 months of age)	Daycare attendance	A different global microbiome profile was observed in the NP microbiome of sOP children when 6 months old *Bacillus*, *Prevotella*, *Gemella*, *Veillonella*, and *Actinomyces* were less abundant in sOP samples The two most important factors associated with these differences were *S. pneumoniae* colonization and daycare attendance
Variability and Diversity of Nasopharyngeal Microbiota in Children: A Metagenomic Analysis (2011) [80]	NP microbiota analysis in healthy children; comparison of microbial composition between children sampled in winter/fall with children sampled in spring	96 healthy children	All samples collected at 18 m of age	Season	Winter samples were characterized by a higher relative abundance of *Proteobacteria* (75% versus 51% in spring) and *Fusobacteria* (14% versus 2% in spring); spring samples had a higher abundance of *Bacteroidetes* (relative abundance: 19% versus 3% in fall/winter), and *Firmicutes*
Dynamics of the nasal microbiota in infancy: A prospective cohort study (2015) [81]	Longitudinal URT microbiota analysis in unselected infants in the first year of life	47 unselected infants (872 total samples collected biweekly)	<1 y	Season	Relative abundance and microbiota composition differed significantly according to season, as *Corynebacteriaceae* were more abundant in summer months, while *Pasteurellacee* were more abundant during winter
Season of Birth Impacts the Neonatal Nasopharyngeal Microbiota (2020) [82]	Analysis of neonatal URT microbiota and its relation with perinatal risk factors	328 samples	1 m	Season	Early NP microbiota is significantly affected by birth season Gram-negative alpha-proteobacteria and Gram-positive Bacilli were more abundant in the nasopharynx of summer-born children

Table 1. *Cont.*

Title (Year of Publication) [Ref]	Study Design	*N.* of Subjects	Age	Environmental Factor	Main Findings
Household siblings and nasal and fecal microbiota in infants (2017) [89]	Cross-sectional analysis of nasal and fecal microbiota and its relation with siblings	105 healthy children	<1 y (median age 3.4 m)	Siblings	Infants with siblings were more likely to have a *Moraxella* dominated profile than *Corynebacterium/Dolosigranulum* dominated profile (76% vs. 18%)
Establishment of the nasal microbiota in the first 18 months of life: Correlation with early-onset rhinitis and wheezing (2018) [90]	Longitudinal nasal microbiota analysis of infants with rhinitis and wheeze in the first 18 months of life and healthy controls	122 children divided in 3 groups: patients with rhinitis alone (n = 28), patients with rhinitis and concomitant wheeze (n = 34), healthy controls (n = 60)	<18 m	Siblings Daycare attendance Delivery route	Control group showed a higher abundance of *Corynebacteriaceae* and early colonization with *Staphylococcaceae* Determinants of nasal microbiota succession included sex, mode of delivery, presence of siblings, daycare attendance
Associations of air pollution and greenness with the nasal microbiota of healthy infants: A longitudinal study (2021) [95]	Longitudinal study investigating the association of greenness and air pollution with the nasal microbiota in the first year of life	47 healthy infants 846 swabs collected	<1 year	Pollution	Distinct microbiota profiles for different PM2.5 exposure levels. Increased NO_2 was associated with reduced abundance of *Corynebacteriaceae*

NP: Nasopharyngeal; URT: Upper respiratory tract; RTI: Respiratory tract infection; IPD: Invasive pneumococcal disease; URTI: Upper respiratory tract infection; ARI: Acute respiratory infection; CS: Caesarian section; sOP: Stringently defined otitis prone; AOM: Acute otitis media.

3.8. Impact of Pneumococcal Vaccination

The introduction of the pneumococcal conjugate vaccination (PCV) has determined an important reduction of OM episodes caused by the serotypes included in the vaccine [97]. A relevant epidemiological study conducted in Sweden on a large cohort has shown that the incidence of AOM has decreased 41.5% and 20.9% in children younger or older than 4 years old, respectively [98]. Similarly, several other epidemiological studies conducted in different countries have reported a reduction in AOM episodes, antibiotic consumption and transtympanic tube placement after the introduction of PCV [99–103]. A Cochrane review by Fortainier et al. evaluated 9 randomized controlled trials including 48.426 children and analyzed the impact on AOM frequency of PCV7, PCV9 and PCV11 conjugated with protein D from *H. influenzae* (PD-PCV11): PCV7 was associated to a reduction of 7% of relative risk if administered in the first year of life, while a higher reduction was identified in relation to AOM specifically caused by *S. pneumoniae* (20% and 52% for PCV7 e PCV11). Moreover, administration of PCV7 was related to a 9–10% reduction in risk of RAOM in children under 1 year of age [104].

The introduction of pneumococcal vaccination programs has induced modifications in OM microbiology, as *H. influenzae* has become the most common otopathogen and serotypes not included in PCVs have been more frequently identified as causative agents of AOM [6,105]. Considering these findings, it is reasonable to speculate that pneumococcal vaccination could impact the composition of the whole bacterial communities in the respiratory system. Unfortunately, evidence available so far on this topic is mostly focused on otopathogens rather than on an analysis of microbiota with high-throughput methods. Moreover, when available, these studies show conflicting results.

Preliminary data have been provided by Hilty et al. in one of the first investigations evaluating the whole URT microbiota in AOM: a previous exposure to PCV7 in children with AOM was associated with a reduced abundance of commensal families such as *Streptococcaceae* and *Corynebacteriaceae* [106]. Biesbroek and colleagues later investigated the impact of PCV7 on URT microbiota by collecting NP swabs from healthy children who received PCV7 and from unvaccinated ones, reporting that vaccination caused a shift in composition and structure of the bacterial community, with an increase of *Veillonella*, *Prevotella*, *Fusobacterium*, *Leptotrichia*, *Actinomyces*, *Rothia*, and nonpneumococcal streptococci [107]. PCV7 and PCV13 and their impact on URT microbiota were further evaluated in a longitudinal study by Mika et al., in which children vaccinated with PCV13 showed a more diverse and stable microbiota and a lower pneumococcal carriage rate compared to those who received PCV7 [108]. On the other hand, it should be noted that other available evidence suggests that PCV might not have a significant influence on the URT microbiota [109–111].

In conclusion, the main findings show that PCVs might have a direct effect on the pneumococcal carriage, with a subsequent possible secondary indirect effect on the whole NP microbiota. However, the difference in pneumococcal carriage rates in relation to geographic region and socio-economic status complicates the interpretation and comparison of the results from different studies, which appear still conflicting so far [112].

4. Discussion

In the URT, *Dolosigranulum* spp. and *Corynebacterium* spp. have been strongly associated with health status and have been defined as potential keystone species in this site [20–23].

Concerning *Dolosigranulum pigrum*, it has been hypothesized that the local production of lactic acid could be related to the lower identification rates of *S. pneumoniae* [113]. However, one of the first detailed studies on this microorganism, conducted by Brugger et al., evidenced that other factors contribute to its anti-pneumococcal activity: possible additional mechanisms proposed by the authors include indeed the competition for nutrients and the production of inhibitory substances, as the genomic analysis of *D. pigrum* strains revealed diverse biosynthetic gene clusters potentially encoding bacteriocins [54]. Moreover, studies conducted on animal models reported a potential immune-modulatory

effect through the activation of Toll-like receptors (TLR-2 and TLR-3) and an induction of enhanced inflammatory response in the first stages of infection through an increased number of neutrophils, macrophages, and higher levels of IL-1β, IFN-γ, and IL-6 [114,115].

Regarding *Corynebacterium*, it has been reported that *C. pseudodiphtericum* is able to competitively exclude *M. catharralis* and improve resistance to RSV and pneumococcal infection in a murine model [116,117]. An interference assay conducted by Lappan et al. in order to assess the inhibitory activity of *C. pseudodiphtericum* and *D. pigrum* against the otopathogens confirmed the ability of *C. pseudodiphtericum* to inhibit *M. catarrhalis* growth; the authors supposed that this effect could be related not to the production of antimicrobial substances, yet to other mechanisms such as competition for nutrients [118].

In our review, the majority of the evidence analyzed showed that environmental risk factors for AOM impair the URT microbiota primarily causing a reduction in the relative abundance of these two potential beneficial microorganisms. Studies available to date are mostly associative and lack a functional analysis of the microorganisms identified as potentially protective; however, in recent years growing evidence is deepening our knowledge of the microbiological aspects, further validating the role of *D. pigrum* and *C. pseudodiphtericum* as key bacteria of the URT.

Taken together, available studies disclose a relevant impact of environmental risk factors on the delicate balance of microbial communities in this region. We can hypothesize that a decreased abundance of potentially protective bacteria, which can be induced by external agents, can favor a higher colonization rate by respiratory pathogens, with a subsequent higher incidence of respiratory disease. Infective episodes, both viral and bacterial, and antibiotic consumption can in turn further sustain this imbalance, predisposing to a vicious cycle of recurrences. Moreover, evidence analyzed in this review shows how certain developmental trajectories and distinct microbiota profiles established since the first phases of life can predispose to respiratory disease at later ages. Environmental risk factors thus likely predispose to AOM incidence also through URT microbiota impairment, starting from birth and the first months of life via delivery route and type of feeding, and at a later time via other agents such as daycare attendance.

5. Limitations

Most of the studies identified in our research are observational, while investigations concerning the causative mechanisms and the cause-effect relations between a certain risk factor and the alteration of microbial communities are lacking. Moreover, the results of different microbiome studies are often difficult to compare because of dissimilarities in specimen collection, analysis, and data reporting.

Most of these studies have been conducted with 16S rRNA PCR, a marker gene analysis that, combined with next-generation sequencing techniques, permits the simultaneous characterization of a whole community. Even if this approach allows a fast and cost-effective analysis, there are also some limitations that should be considered: there is limited functional information, as it is not possible to determine whether taxa detected are active or inactive; low biomass samples are susceptible to over-amplification bias; the taxonomic resolution is usually limited to family or genus level. Full-gene 16S rRNA gene sequencing and metagenome and metatrascriptome analysis may overcome some of these limitations but are less adopted as they are relatively expensive and complex to perform.

6. Conclusions

The introduction of novel high-throughput techniques has changed our approach to the investigation of infectious diseases and has provided novel insights into AOM pathogenesis, through the identification of potential protective genera in the URT. Evidence shows a potential impact of the most relevant environmental risk factors on the overall composition of microbial communities in the URT and on the relative abundance of potential keystone genera. Deepening our knowledge of AOM epidemiology and pathogenesis under the microbiome perspective could be of remarkable importance to improve our

preventive strategies, in particular through the development of probiotic therapies. In our opinion, further investigations should be focused on the following aspects:

- Deepening our knowledge on the impact of both risk and protective factors, such as vaccinations
- Exploring the cause-effective correlation between environmental agents and subsequent microbial modifications
- Focusing on Corynebacterium and Dolosigranulum role in the URT, in order to define their possible use as probiotics.

Author Contributions: F.F. conceived and wrote the manuscript; P.B. conducted the literature search and contributed to drafting the manuscript; P.M., S.T. and M.G. revised the paper for important intellectual contribution. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest.

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