

Review Article

Asian Pacific Journal of Tropical Medicine

doi: 10.4103/apjtm.apjtm_334_26

Pediatric upper-airway microbiota and respiratory infections in tropical low- and middle-income settings: A narrative review and low-biomass research framework

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ABSTRACT

Pediatric upper-airway microbiota has been increasingly linked to respiratory-health phenotypes, but the transferability of these signals to low- and middle-income countries (LMICs) remains uncertain. Most phenotype-specific evidence comes from high-income settings, where diagnostic capacity, antibiotic exposure, vaccination coverage, environmental risks, and sample-handling conditions may differ substantially from LMIC contexts. This narrative review synthesizes current evidence on pediatric nasal and nasopharyngeal microbiota, respiratory-health phenotypes, LMIC contextual modifiers, and low-biomass microbiome methodology. Original studies linking upper-airway microbiota patterns with clinically defined respiratory outcomes were mapped and synthesized thematically. Across studies, respiratory-health signals were better characterized as phenotype-dependent community shifts than as disease-specific organisms. *Corynebacterium*- and *Dolosigranulum*-enriched profiles were commonly associated with more stable or lower-risk respiratory states, whereas *Haemophilus*-, *Streptococcus*-, and *Moraxella*-dominant profiles recurred across infection-associated and inflammatory phenotypes. In LMIC settings, these signals may be modified by pre-sampling antibiotics, repeated infectious exposure, household crowding, environmental pollution, nutritional vulnerability, vaccination heterogeneity, diagnostic uncertainty, and transport or storage constraints. Interpretable low-biomass research requires standardized sampling, contamination controls, transparent 16S rRNA workflows, and context-rich metadata. Pediatric upper-airway microbiota should therefore be interpreted as a context-dependent ecological signal rather than an immediate diagnostic biomarker. Future LMIC studies should prioritize phenotype-specific hypotheses, rigorous but feasible low-biomass workflows,

and locally relevant metadata to support respiratory phenotyping, antibiotic-stewardship research, vaccine-effect evaluation, and child-health interventions.

KEYWORDS: Pediatric microbiota; Nasopharyngeal microbiome; Respiratory infections; 16S rRNA; Low-biomass microbiome; Low- and middle-income countries

1. Introduction

Respiratory illnesses remain a major cause of childhood morbidity worldwide, encompassing acute upper and lower respiratory tract infections, recurrent respiratory morbidity, otitis media, and allergic or inflammatory airway disorders[1,2]. In early life, the upper airway is a dynamic ecological interface where microbial colonization, viral exposure, mucosal immune maturation, environmental conditions, and medical interventions interact to shape respiratory-health trajectories[3,4].

The nasopharynx is particularly relevant because it functions as both a reservoir for respiratory pathobionts and a site of ecological

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How to cite this article: Quang NC, Thuy NTD, Hung TD, Tu TT, Dang THH. Pediatric upper-airway microbiota and respiratory infections in tropical low- and middle-income settings: A narrative review and low-biomass research framework. Asian Pac J Trop Med 2026; 19(9): 380-391.

Article history: Received 26 June 2026

Revision 2 September 2026

Accepted 9 September 2026

Available online 14 September 2026

competition among commensal and potentially pathogenic organisms[4,5]. Sequencing-based studies link *Corynebacterium/Dolosigranulum*-enriched profiles with more stable respiratory states, while *Haemophilus*, *Streptococcus* and *Moraxella* enrichment recurs in infection-associated and inflammatory phenotypes. These profiles should be interpreted as ecological signals, not diagnostic biomarkers[3-7]. However, most phenotype-specific evidence comes from high-income settings, creating a transferability problem for low- and middle-income countries (LMICs). In resource-constrained settings, microbiota signals may be shaped by high respiratory morbidity, empirical antibiotic use, household crowding, pollution, nutritional vulnerability, heterogeneous vaccination coverage, limited diagnostics, care-seeking pathways, and sample-handling constraints[8]. Interpretation is further complicated by the low-biomass nature of nasal and nasopharyngeal specimens, where pre-analytic variation, contamination, incomplete metadata, logistical constraints, and empirical treatment before sampling may distort observed community patterns[9].

This narrative review synthesizes phenotype-specific evidence on pediatric upper-airway microbiota and respiratory health to develop an LMIC-oriented framework for contextual interpretation and low-biomass research. It aims to summarize recurring microbiota signals across pediatric respiratory phenotypes, examine how LMIC contextual factors may modify their interpretation, and outline pragmatic priorities for designing interpretable microbiota studies in resource-constrained settings.

2. Literature review

2.1. Review design and scope

We conducted a narrative review of current evidence on pediatric upper-airway microbiota and respiratory health. This article was designed to synthesize phenotype-specific microbiota evidence and interpret its transferability to LMIC settings, rather than to estimate pooled effects, assess intervention effectiveness, or formally grade certainty of evidence. Existing evidence was used as a biological foundation to examine how microbiota–respiratory phenotype signals may be interpreted, adapted, and operationalized for LMIC research contexts.

The review was organized around three linked components: phenotype-specific microbiota evidence, LMIC-oriented contextual interpretation, and methodological priorities for low-biomass microbiota studies. Original studies were assessed for reported microbiota–phenotype associations, contextual variables, and methodological choices related to sampling, sequencing, contamination control, bioinformatics, and metadata.

2.2. Search strategy and evidence domains

Targeted, iterative searches were performed in PubMed/MEDLINE, Scopus, Embase, and Google Scholar for English-language literature relevant to three areas: phenotype-specific pediatric upper-airway microbiota evidence, contextual factors that may affect transferability to LMIC research settings, and low-biomass microbiome methodology. The final search was updated on 15 March 2026. Reference lists of relevant reviews and key original studies were also screened to identify additional publications.

Searches combined terms related to population, sampling site, methodology, respiratory phenotype, and context, including “child”, “infant”, “pediatric”, “nasopharyngeal microbiota”, “nasal microbiota”, “upper airway microbiome”, “16S rRNA”, “low biomass”, “contamination”, “upper respiratory tract infection”, “bronchiolitis”, “RSV”, “pneumonia”, “wheezing”, “asthma”, “acute otitis media”, “pneumococcal disease”, “allergic rhinitis”, “LMIC”, “low-resource settings”, “antibiotics”, “vaccination”, “air pollution”, “nutrition”, “household crowding”, “sample transport”, and “cold chain”.

Evidence was grouped into three domains: original pediatric studies linking upper-airway microbiota with respiratory phenotypes; literature on LMIC contextual modifiers; and methodological literature on low-biomass respiratory microbiota research, including sampling, contamination control, 16S rRNA sequencing, and bioinformatics reproducibility.

2.3. Selection of evidence and evidence mapping

For the phenotype-specific synthesis, we prioritized original pediatric studies using sequencing-based nasal or nasopharyngeal microbiota profiling and directly linking microbiota patterns with clinically defined respiratory outcomes. The evidence map was organized around major respiratory-health categories, including healthy maturation or respiratory stability, viral upper respiratory tract infection, bronchiolitis/RSV disease, pneumonia or severe lower respiratory tract infection, viral wheeze, asthma-related illness, acute otitis media, pneumococcal disease, and allergic rhinitis.

Studies were excluded from the phenotype evidence map if they were adult-only, non-human or *in vitro* studies, review articles, studies without sequencing-based microbiota profiling, studies focused on non-respiratory microbiomes, or studies examining microbiota determinants without respiratory-health outcomes. The latter were considered only when they informed contextual interpretation or methodological priorities.

A selected evidence map of original studies was prepared to support the narrative synthesis. For each study, we extracted country or setting, study design, age group, sampling site, respiratory phenotype, main microbiota finding, and rationale for inclusion.

Supplementary Table 1 was restricted to phenotype-specific evidence rather than exhaustive methodological extraction. The literature identification and selection workflow is summarized in Figure 1.

2.4. Narrative synthesis and interpretation

Findings were synthesized thematically rather than pooled quantitatively because of heterogeneity in respiratory phenotypes,

sampling sites, sequencing targets, bioinformatics pipelines, exposure measurement, outcome definitions, and health-system contexts. Evidence was grouped by respiratory phenotype, and recurring microbiota patterns were interpreted alongside contextual variables and methodological considerations relevant to LMIC research settings.

Respiratory phenotypes were assigned a qualitative level of relative consistency of evidence based on recurrence of microbiota patterns

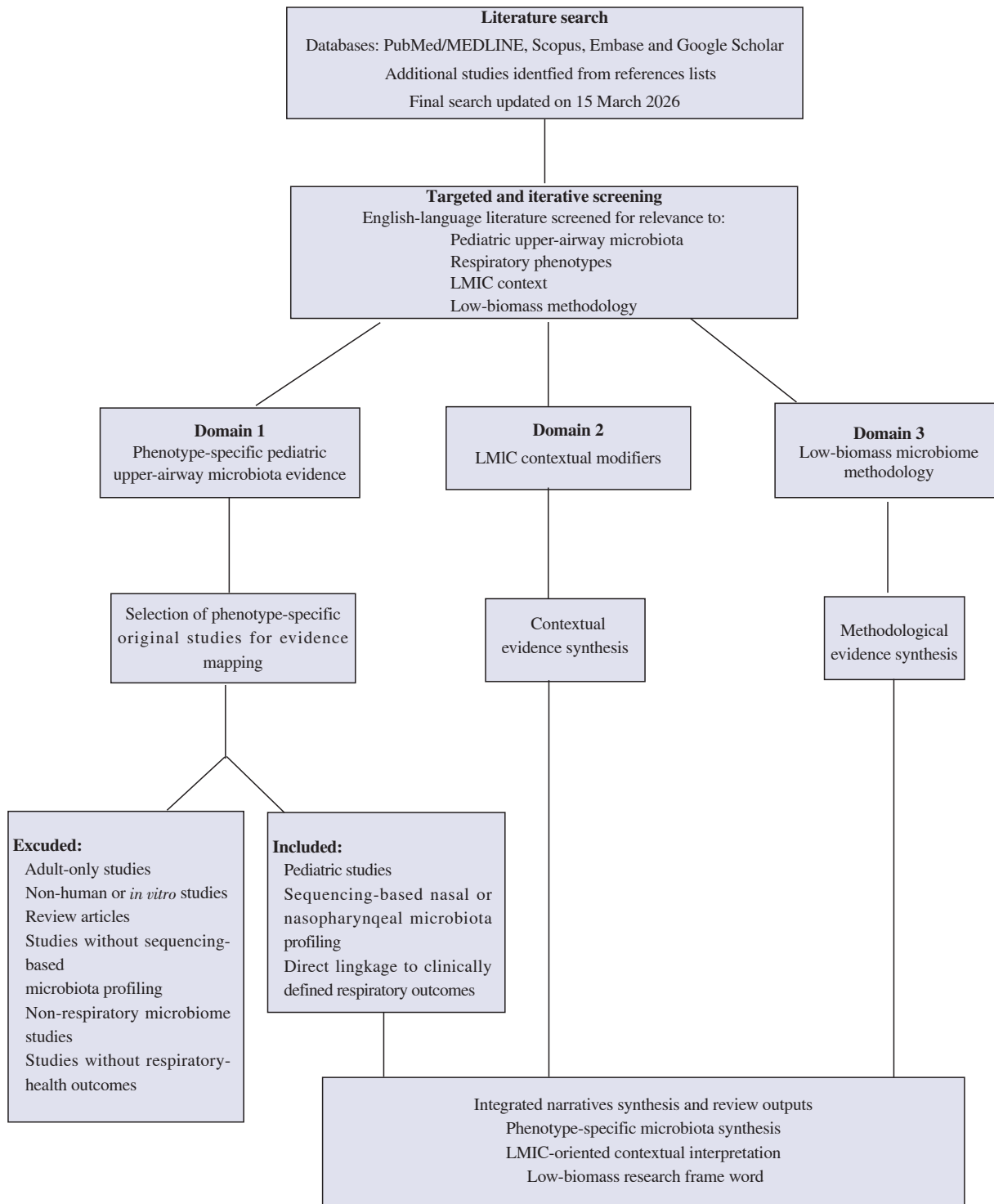


Figure 1. Literature identification and selection workflow for the narrative review. LMIC: low- and middle-income country.

across studies, phenotype specificity, availability of longitudinal or disease-specific evidence, adjustment for clinical covariates, and biological plausibility. This rating was intended only to guide narrative interpretation and should not be considered a formal certainty-of-evidence assessment.

3. Phenotype-specific upper-airway microbiota signals in pediatric respiratory health

3.1. Overview of selected original studies

These studies were selected for direct relevance to the review framework, not to provide an exhaustive catalogue of pediatric upper-airway microbiome research[3,6,7,10-33]. The selected evidence consisted mainly of birth or infant cohorts, longitudinal pediatric cohorts, case-control studies, and disease-comparison studies. Most phenotype-specific and longitudinal evidence came from high-income settings in Europe, North America, and Australia, with additional studies from East Asia and selected underrepresented or low-resource settings. This distribution highlights a transferability gap because microbiota signals are often derived from settings that differ from LMIC contexts in diagnostic capacity, vaccination, antibiotic use, and laboratory infrastructure. Across studies, respiratory-health signals were better characterized as phenotype-dependent community shifts than as single disease-specific organisms (Table 1). This supports a synthesis organized by respiratory phenotype and interpreted alongside key clinical, epidemiological, and methodological contexts.

3.2. Healthy maturation and ecological stability

During infancy and early childhood, nasopharyngeal communities undergo rapid succession from unstable early-life communities toward more organized community states shaped by host, environmental, infectious, and early childhood, nasopharyngeal communities undergo rapid succession from unstable early-life communities and healthcare-related exposures[3,4,6]. Across cohorts, *Corynebacterium*- and *Dolosigranulum*-enriched communities are repeatedly described as relatively stable or lower-risk profiles, possibly reflecting colonization resistance, microbial competition, or modulation of local inflammatory responses[3,6,28]. However, these taxa should not be treated as universal “healthy microbiota” signatures, because their meaning depends on age, local ecology, exposure history, and broader community structure[3,6,25].

3.3. Acute respiratory infections and viral-bacterial interactions

In acute respiratory infections, the most consistent signal is a shift toward communities enriched with potential pathobionts, particularly *Haemophilus*, *Streptococcus*, and *Moraxella*. These

Table 1. Pediatric upper-airway microbiota signals across respiratory-health phenotypes and LMIC-oriented interpretation.

Respiratory phenotype	Sampling site/evidence base	Recurrent microbiota signal	Clinical/ecological interpretation	Relative consistency of evidence	LMIC-oriented interpretation	Key references
Healthy maturation/ ecological stability	Mainly nasopharyngeal samples; infant and early-childhood cohorts	<i>Corynebacterium</i> - and <i>Dolosigranulum</i> -enriched communities; <i>Moraxella</i> may also appear during age-dependent maturation	Suggests a more stable or resilient upper-airway ecosystem rather than a universal “healthy” microbiota signature	Moderate	Age, season, breastfeeding, antibiotic exposure, vaccination history, and sampling timing should be captured to distinguish normal maturation from illness- or treatment-related perturbation	Bosch <i>et al.</i> [3]; Biesbroek <i>et al.</i> [6]
Viral acute respiratory infection/URTI	Mainly nasopharyngeal samples; longitudinal infant studies and infection-associated sampling	Transient expansion or dominance of <i>Streptococcus</i> , <i>Moraxella</i> , or <i>Haemophilus</i> during virus-associated illness	Supports a virus-bacteria-host interaction model rather than a virus-only disease model	Moderate	Repeated viral exposure, household crowding, daycare or school attendance, and pre-sampling antibiotics may modify observed community states	Teo <i>et al.</i> [7]; Chonmaitree <i>et al.</i> [11]; McCauley <i>et al.</i> [12]; Wen <i>et al.</i> [31]
Bronchiolitis/RSV disease	Nasopharyngeal samples; hospitalized infant bronchiolitis and RSV cohorts	<i>Haemophilus</i> -dominant or <i>Haemophilus</i> -enriched profiles; association with severity, higher viral load, mucosal CXCL8 response, or delayed RSV clearance	<i>Haemophilus</i> dominance may mark a more inflammatory or less resilient airway ecosystem during severe viral lower respiratory disease	Relatively high	Interpretation should account for delayed presentation, empirical antibiotics, viral testing availability, referral patterns, and hospitalization thresholds	Hasegawa <i>et al.</i> [13]; Ederveen <i>et al.</i> [14]; Mansbach <i>et al.</i> [15]

Table 1 continued on the next page.

Table 1. Continued.

Respiratory phenotype	Sampling site/evidence base	Recurrent microbiota signal	Clinical/ecological interpretation	Relative consistency of evidence	LMIC-oriented interpretation	Key references
Pneumonia/severe lower respiratory tract infection	Mainly nasopharyngeal or nasal samples; selected studies included paired upper/lower-airway or oropharyngeal samples when the upper-airway component informed phenotype interpretation	Altered upper-airway microbiota in pneumonia or severe LRTI phenotypes; pathobiont enrichment and community disruption reported across pneumonia cohorts; upper-lower airway concordance reported in selected MPP cohorts	Upper-airway microbiota may reflect pathogen reservoir, colonization pressure, host susceptibility, or upper-lower airway ecological linkage rather than direct evidence of lung infection	Low to moderate	Highly relevant in LMICs because pre-hospital antibiotics, delayed care-seeking, limited pathogen diagnostics, referral bias, and vaccine heterogeneity may strongly affect microbiota–pneumonia interpretation	Man <i>et al.</i> [17]; de Koff <i>et al.</i> [18]; Claassen-Weitz <i>et al.</i> [21]; Makori <i>et al.</i> [22]; Lu <i>et al.</i> [23]; Dai <i>et al.</i> [24]
Rhinovirus-associated wheezing/viral wheeze	Upper-airway/nasopharyngeal samples; early-life viral illness and wheezing cohorts	Pathobiont-rich profiles involving <i>Streptococcus</i> , <i>Moraxella</i> , or <i>Haemophilus</i> ; less consistent rhinovirus-specific genus pattern than RSV	Supports bacterial–viral interaction, but rhinovirus-specific microbiota signatures remain less settled	Low to moderate	Requires viral typing, timing of sample collection, and distinction between asymptomatic carriage, viral illness, and symptomatic wheeze	Teo <i>et al.</i> [7]; Teo <i>et al.</i> [10]
Wheezing/asthma susceptibility	Mainly nasopharyngeal and upper-airway samples; prospective infant cohorts and asthma-focused studies	Early-life <i>Streptococcus</i> colonization; repeated expansions of <i>Streptococcus</i> , <i>Moraxella</i> , or <i>Haemophilus</i> during respiratory illness	Early dysbiosis and repeated airway perturbation may identify high-risk respiratory trajectories rather than a single asthma-specific microbiota	Moderate	Interpretation should integrate allergic sensitization, viral infection, antibiotic exposure, indoor pollution, socioeconomic context, and timing of sampling	Teo <i>et al.</i> [7]; Teo <i>et al.</i> [10]; Hou <i>et al.</i> [30]
Allergic rhinitis/allergic airway disease	Mostly nasal rather than strictly nasopharyngeal samples; pediatric allergic-rhinitis studies	<i>Heterogeneous</i> findings; altered diversity/evenness and selected taxa reported in some studies	Emerging area; no robust universal nasal or nasopharyngeal microbiota signature has been established	Emerging/limited	Should be interpreted cautiously; sampling site, allergen exposure, indoor environment, pollution, and local environmental microbiota are critical	Teng <i>et al.</i> [32]; Tang <i>et al.</i> [33]
Acute otitis media	Nasopharyngeal samples; infant URTI/AOM cohorts	Shifts involving <i>Streptococcus</i> , <i>Haemophilus</i> , and <i>Moraxella</i> around viral URTI and AOM episodes	Supports the nasopharynx as a reservoir and ecological source niche for middle-ear infection	Moderate	Important where antibiotic exposure before diagnosis may alter otopathogen ecology and reduce interpretability of culture or PCR results	Chonmaitree <i>et al.</i> [11]
Pneumococcal disease/severe bacterial phenotype	Nasopharyngeal samples; case-control or disease-comparison studies	<i>Streptococcus pneumoniae</i> enrichment with depletion of potentially stabilizing taxa such as <i>Dolosigranulum</i> in some studies	Severe bacterial disease may reflect both pathogen enrichment and loss of ecological competitors or stabilizing community members	Moderate	PCV coverage, serotype replacement, viral co-infection, and antibiotic exposure should be incorporated into interpretation	Henares <i>et al.</i> [25]; Camelo-Castillo <i>et al.</i> [26]

Notes: The level of relative consistency reflects a qualitative judgment based on recurrence of microbiota patterns across studies, phenotype specificity, availability of longitudinal or disease-specific evidence, adjustment for clinical covariates, and biological plausibility. It is not intended as a formal GRADE or certainty-of-evidence assessment. AOM: acute otitis media; LMIC: low- and middle-income country; LRTI: lower respiratory tract infection; MPP: *Mycoplasma pneumoniae* pneumonia; PCV: pneumococcal conjugate vaccine; RSV: respiratory syncytial virus; URTI: upper respiratory tract infection.

genera are repeatedly reported during viral upper respiratory tract infections, acute otitis media episodes, and infection-associated airway perturbations[7,10–12,31]. This supports a virus–bacteria–host interaction model, in which bacterial community structure may modify viral spread, mucosal inflammation, and clinical severity. In LMIC settings, these transitions should be interpreted in relation to local exposure and treatment contexts[27,28].

3.4. Bronchiolitis, RSV infection, and disease severity

The strongest phenotype-specific evidence comes from bronchiolitis and RSV-related disease. In infants hospitalized with bronchiolitis, *Haemophilus*-dominant nasopharyngeal profiles have been associated with more severe clinical phenotypes. In RSV infection, *Haemophilus* enrichment has been linked to higher viral load, enhanced mucosal CXCL8 responses, and delayed RSV clearance after adjustment for relevant clinical factors. These findings suggest that *Haemophilus* dominance may identify an inflammatory or less resilient airway ecosystem during severe viral lower respiratory disease[13–15]. However, this should not be interpreted as evidence that *Haemophilus* alone causes severe bronchiolitis; rather, it likely reflects a broader ecological state involving viral infection, mucosal inflammation, host susceptibility, and reduced community resilience.

3.5. Pneumonia and severe lower respiratory tract infection

Pneumonia and severe lower respiratory tract infection remain more difficult to interpret than bronchiolitis or viral wheeze in pediatric upper-airway microbiota research. Nasal and nasopharyngeal samples do not directly measure the lower-airway microbiota; therefore, findings should be interpreted as markers of colonization pressure, pathogen reservoir, host susceptibility, or upper–lower airway ecological linkage rather than direct evidence of lung infection. Across pediatric pneumonia and lower respiratory tract infection studies, altered upper-airway community structure, pathobiont enrichment, and disrupted community profiles have been reported, although patterns vary by pneumonia phenotype, pathogen testing, disease severity, and sampling strategy[17–22]. In selected cohorts, including studies of *Mycoplasma pneumoniae* pneumonia, concordance between upper- and lower-respiratory microbiota has also been described, supporting cautious interpretation of upper-airway signals as part of a broader respiratory ecosystem[23,24]. For LMIC-oriented interpretation, pneumonia-focused microbiota findings should be read in relation to local diagnostic capacity, treatment pathways, vaccination context, and timing of sampling rather than treated as direct microbial evidence of lower-airway infection[27,28].

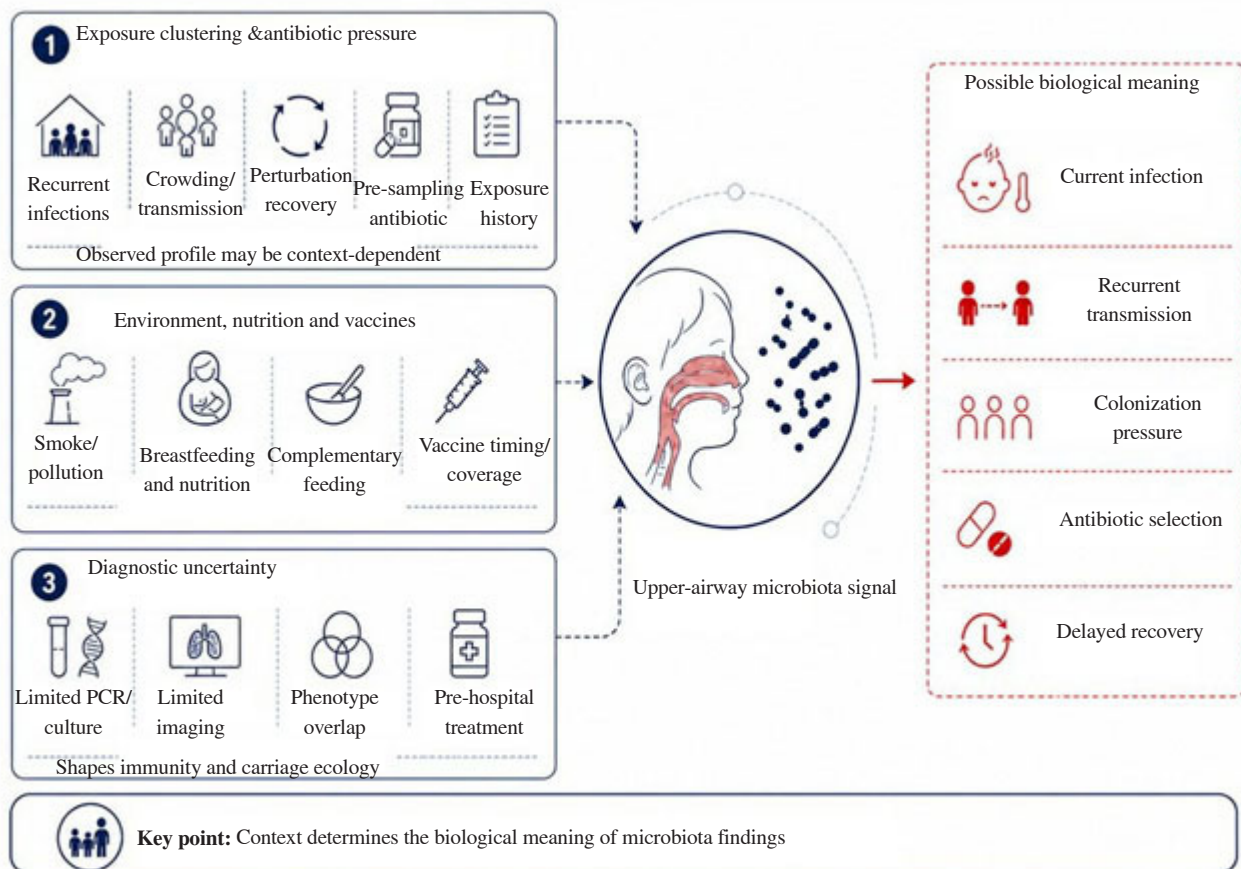


Figure 2. LMIC-related contextual modifiers that may alter interpretation of pediatric upper-airway microbiota signals across respiratory phenotypes. LMIC: low- and middle-income countries.

3.6. Wheezing, asthma susceptibility, and allergic airway disease

For wheezing, asthma-related outcomes, and allergic airway disease, evidence points less toward fixed disease-specific microbiota signatures and more toward early-life dysbiosis, infection-associated perturbations, and interactions among bacterial communities, viral infection, and allergic sensitization[7,10,30]. Early-life enrichment of *Streptococcus*, *Moraxella*, or *Haemophilus* has been linked to wheezing or asthma-related trajectories, whereas pediatric allergic rhinitis studies remain more heterogeneous and rely mainly on nasal samples[32,33]. Interpretation should therefore account for age, viral context, allergic sensitization, sampling site, and environmental exposure.

3.7. Otitis media and pneumococcal disease

The nasopharynx serves as an ecological reservoir for otopathogens and pneumococcal disease. In acute otitis media, infant microbiota shifts often involve *Streptococcus*, *Haemophilus*, and *Moraxella*, supporting the nasopharynx as a source niche for middle-ear infection during or after viral upper respiratory tract infection (URTI)[11,29]. For pneumococcal disease, interpretation requires caution because 16S rRNA sequencing may not reliably distinguish commensal streptococci from pathogenic *Streptococcus pneumoniae*. Invasive pneumococcal disease studies suggest that severe bacterial phenotypes may involve pathogen enrichment and depletion of stabilizing taxa such as *Dolosigranulum*, supporting an ecological model of disease risk[25,26].

3.8. Integrative interpretation for LMIC respiratory research

Overall, phenotype-specific evidence supports an ecological rather than diagnostic interpretation of pediatric upper-airway microbiota. Recurrent community patterns provide a biological basis for LMIC-oriented interpretation, but their meaning varies by clinical phenotype, illness timing, treatment history, sampling site and local epidemiology. Therefore, in LMIC respiratory research, microbiota signals should be interpreted together with diagnostic capacity, antibiotic exposure, environmental and nutritional context, and sample-handling conditions.

4. Why LMIC context modifies the interpretation of pediatric upper-airway microbiota

LMIC context does not imply that antibiotics, infectious exposure, air pollution, nutrition, or vaccination are unique to LMICs. Rather, these factors may differ in prevalence, intensity, clustering, timing,

and measurement quality. In resource-constrained contexts, high respiratory morbidity often coexists with empirical antibiotic use, delayed care-seeking, household crowding, pollution, nutritional vulnerability, heterogeneous vaccination coverage, limited diagnostics, and sample-handling constraints. These factors may affect both the microbiota itself and the external validity of microbiota–phenotype associations derived from high-income settings (Figure 2)[21,22,28,34].

4.1. Exposure clustering and antibiotic pressure

In LMIC settings, respiratory exposures often cluster rather than occur in isolation. Repeated infections, household transmission, crowding and healthcare encounters may increase perturbation–recovery cycles in the upper-airway microbiota, so a pathobiont-enriched profile during illness may reflect current infection, recent transmission, persistent carriage pressure, prior treatment or delayed ecological recovery rather than a single disease-specific signal[27,28]. Pre-sampling antibiotic exposure is particularly important because treatment may occur before clinical phenotyping or microbiota collection. This can alter community structure and blur microbiota–disease associations: taxon depletion may reflect treatment effect, whereas persistence of selected organisms may reflect antimicrobial selection or post-treatment recovery. LMIC studies should therefore record antibiotic use before sampling, timing of the last dose, drug class, indication and recent cumulative exposure[27,28,34].

4.2. Environmental, nutritional, and vaccine-related heterogeneity

Environmental, nutritional and vaccine-related factors may modify the biological meaning of upper-airway microbiota signals. Smoke and pollution exposure, poor ventilation, nutritional vulnerability and breastfeeding patterns can influence respiratory susceptibility and may co-occur with infection burden and antibiotic use in LMIC settings[34]. These factors should therefore be considered as contextual modifiers rather than isolated covariates. Vaccination is also important because pneumococcal conjugate vaccines and other childhood vaccines can alter carriage ecology and bacterial competition. Vaccine type, dose timing and local coverage should be captured whenever possible to avoid misinterpreting vaccine- or population-related carriage differences as disease-specific microbiota shifts[6].

4.3. Diagnostic uncertainty and phenotype misclassification

A major LMIC-related challenge is that microbiota interpretation depends on the quality of respiratory phenotype classification. Limited access to viral PCR, bacterial diagnostics, radiography, oxygenation assessment and standardized follow-up can make

categories such as viral URTI, bronchiolitis and pneumonia clinically heterogeneous[9,35,36]. Pre-hospital antibiotics, delayed presentation, and referral bias may further obscure whether a microbiota profile reflects viral illness, bacterial disease, mixed infection, or treatment-related perturbation. Thus, the same *Haemophilus*-dominant profile may represent different biological states depending on context. Microbiota findings should therefore be interpreted together with phenotype validity, antibiotic timing, diagnostic certainty, care pathway, and sample-handling conditions.

5. Implementing interpretable low-biomass nasopharyngeal microbiota research in LMICs

If LMIC context changes interpretation, study design must make microbiota signals both technically credible and clinically interpretable. This is particularly important for nasal and nasopharyngeal samples, where low microbial biomass makes results vulnerable to pre-analytic variation, contamination, batch effects and incomplete metadata[9,36,37]. In LMIC settings, these risks may be amplified by logistical constraints, external sequencing, empirical treatment before sampling and limited diagnostic information. The priority is therefore pragmatic standardization: a workflow rigorous enough to protect biological signal validity, but feasible enough to be implemented consistently in resource-constrained pediatric settings (Table 2).

5.1. From low-biomass standards to pragmatic LMIC workflows

For many LMIC pediatric respiratory studies, 16S rRNA gene sequencing remains a practical entry platform because it is less expensive and more scalable than shotgun metagenomics. However, its limitations must be explicit. It usually provides limited species- or strain-level resolution and does not directly capture viruses, fungi, antimicrobial-resistance genes, or functional pathways[9,36,37]. A *Streptococcus* signal should not automatically be interpreted as *Streptococcus pneumoniae*, and a *Haemophilus* signal may not distinguish pathogenic from commensal species. When pathogen-level inference is central to the clinical question, 16S should be complemented by targeted PCR, culture, serotyping, or metagenomics where feasible.

Because nasal and nasopharyngeal samples are low-biomass specimens, variation in sampling, transport, storage, extraction batch, contamination, or sequencing pipeline can affect observed profiles[9,36]. Studies should define a feasible minimum workflow, document deviations, include negative controls, and report core sequencing and bioinformatics parameters. Methodological rigor is therefore a prerequisite for valid interpretation of microbiota-respiratory phenotype associations[3,6,7,11,12,23,27,30,32,33].

5.2. Context-rich metadata and feasible study designs

Technical standardization alone is insufficient if clinical and contextual data are weak. In LMIC pediatric respiratory research, microbiota signals may reflect disease biology, treatment effect, exposure history, or phenotype misclassification. A minimum metadata set should include age, sex, respiratory phenotype, illness timing, antibiotic exposure, vaccination history, nutrition, smoke or pollution exposure, household crowding, season, illness severity, and sampling conditions[3,6,17,27,28].

Study design should also match the biological question and local feasibility. Large cross-sectional studies may appear efficient, but can be difficult to interpret when children are sampled at different illness stages or after variable antibiotic exposure. Smaller longitudinal cohorts with repeated sampling before illness, during acute symptoms, and after recovery may better capture microbiota transitions. Nested case-control studies within well-characterized cohorts can reduce sequencing costs while preserving interpretability. For pneumonia or bronchiolitis, sentinel hospital-based cohorts with standardized severity assessment, documented pre-sampling antibiotic exposure, and available viral testing may be more informative than broad but poorly annotated surveys[3,6,21,22,27,38].

A practical principle for LMIC research is that fewer well-characterized samples may be more valuable than many poorly annotated samples. The goal is not the most advanced sequencing platform, but microbiota data that can be interpreted alongside clinical phenotype, exposure history, treatment pathway, and sample quality. This approach can support context-aware respiratory phenotyping, antibiotic-stewardship research, vaccine-effect evaluation, and locally relevant child-health interventions within resource-constrained health systems.

6. Discussion

This narrative review reframes pediatric upper-airway microbiota evidence through an LMIC-oriented interpretive and implementation lens. Its main contribution is to clarify how phenotype-specific microbiota signals, largely generated in high-income settings, should be interpreted and studied in resource-constrained pediatric contexts[3,6,7,11,12,25,27,28].

The synthesis supports an ecological rather than diagnostic interpretation of nasal and nasopharyngeal microbiota. Similar taxonomic patterns may represent different biological states depending on age, illness timing, viral co-detection, treatment history, sampling site, and local pathogen ecology[3,6,17,28]. Microbiota findings should therefore be interpreted as context-dependent ecological readouts rather than stand-alone disease labels. The main challenge for LMIC translation is external validity. Existing evidence remains biologically informative, but its

Table 2. LMIC-adapted priorities for interpretable low-biomass nasopharyngeal microbiota studies.

Domain	Why it is especially important in LMIC settings	Minimum pragmatic approach	Interpretation risk if omitted
Phenotype definition under diagnostic uncertainty	Viral PCR, bacterial diagnostics, radiography, and standardized follow-up may be inconsistently available	Use simple, reproducible clinical definitions; record available diagnostics and severity indicators	Microbiota signals may reflect mixed or misclassified phenotypes rather than true disease-specific patterns
Sampling site and collection standardization	Nasal and nasopharyngeal sampling may vary by collector training, equipment availability, and clinical workflow	Standardize sampling site, swab type, collection procedure, collector training, and number of sampling attempts	Differences in sampling technique may be misinterpreted as biological or phenotype-related microbiota variation
Pre-sampling antibiotic exposure	Antibiotics may be used empirically, before referral, or without prescription before microbiota sampling	Record antibiotic use before sampling, timing of last dose, drug class, indication, and cumulative recent exposure	Pathobiont depletion or persistence may be mistaken for disease biology rather than treatment effect
Timing of sampling in delayed care-seeking	Children may present after several days of illness or after initial treatment elsewhere	Record days from symptom onset, prior healthcare contact, and timing relative to antibiotics	Acute, recovery, and post-treatment communities may be mixed together
Cold-chain and transport feasibility	Samples may be collected far from sequencing laboratories, with unstable electricity or delayed transport	Use validated transport media or cold-chain SOPs; record transport time, temperature, and deviations	Technical variation may be misinterpreted as geographic, setting-related, or disease-related microbiota differences
Low-biomass contamination control	Low microbial biomass, variable laboratory infrastructure, and external sequencing arrangements may increase vulnerability to contamination or batch effects	Include extraction blanks, reagent controls, and field controls where feasible; report contaminant filtering	Reagent, handling, or batch-related contaminants may be interpreted as true low-abundance respiratory taxa
Contextual exposure clustering	Crowding, repeated infection, smoke, pollution, nutrition, and antibiotic exposure often co-occur	Collect a short core metadata set: household density, siblings, daycare/school, smoke/cooking fuel, nutrition, breastfeeding, and season	Apparent microbiota–phenotype associations may actually reflect clustered exposures
Vaccination and local carriage ecology	PCV, Hib, and influenza vaccine coverage, timing, catch-up schedules, and herd effects may vary across communities	Record vaccine type, doses, timing relative to sampling, catch-up status, and local coverage where possible	Differences in carriage ecology may be misread as disease-specific microbiota shifts
16S rRNA limitations in pathogen inference	In settings with limited diagnostic testing, there may be temptation to use 16S rRNA sequencing as pathogen identification	State taxonomic resolution; avoid equating genus-level signals with pathogens; complement with PCR, culture, or serotyping when feasible	<i>Streptococcus</i> or <i>Haemophilus</i> signals may be overinterpreted as direct etiologic agents
Bioinformatics transparency and reproducibility	External or collaborative analysis may use different pipelines, databases, and filtering thresholds	Report software, versions, denoising approach, taxonomy database, read-depth thresholds, contaminant filtering, and batch handling	Analytical choices may masquerade as phenotype, setting, or country differences
Batching and external sequencing	Samples may be shipped and processed in batches due to cost or logistics	Balance phenotype groups across extraction and sequencing batches; document batch structure	Batch effects may masquerade as phenotype or setting differences
Feasible and interpretable study design	Large longitudinal studies may be costly, but cross-sectional designs are vulnerable to timing and antibiotic bias	Use small repeated-sampling cohorts, nested case-control designs, or sentinel hospital cohorts with rich metadata	Large but poorly annotated datasets may be less interpretable than smaller well-characterized studies

Note: These priorities are not intended as a generic microbiome checklist. They highlight LMIC-adapted considerations where diagnostic uncertainty, pre-sampling antibiotic exposure, exposure clustering, transport constraints, low-biomass contamination, and measurement feasibility may alter the interpretation of nasopharyngeal or nasal microbiota data. These priorities were informed by low-biomass microbiome methodology, pediatric upper-airway microbiota studies, and 16S rRNA sequencing/reporting considerations. Hib: *Haemophilus influenzae* type b; LMIC: low- and middle-income country; PCV: pneumococcal conjugate vaccine; SOP: standard operating procedure; 16S rRNA: 16S ribosomal RNA.

applicability cannot be assumed when antibiotic exposure, infectious pressure, household crowding, environmental pollution, nutrition, vaccination coverage, diagnostic access, and care pathways differ across settings[21,22,27-29]. Future studies should examine how microbiota signals are reshaped by local epidemiology, treatment practices, diagnostic capacity, and health-system constraints.

Methodologically, advanced sequencing cannot compensate for weak phenotype definition, undocumented antibiotic exposure, inadequate contamination control, or incomplete metadata. Smaller but well-characterized cohorts may generate more interpretable evidence than larger poorly annotated datasets. Standardized sampling, negative controls, transparent *16S* rRNA workflows, batch documentation, and core contextual metadata are prerequisites for credible interpretation[9,36].

The practical implication is that LMIC-oriented pediatric upper-airway microbiota studies should be designed around clear respiratory phenotypes and locally relevant decision problems. For example, microbiota data may be useful for refining respiratory phenotype classification, understanding antibiotic-related ecological perturbation, evaluating vaccine-related carriage changes, or identifying children with recurrent respiratory susceptibility. However, these applications require careful linkage between microbiota profiles, clinical timing, treatment history, diagnostic certainty and environmental context. Microbiota should therefore be positioned as a tool for contextual respiratory-health research rather than as an immediate diagnostic test.

This review has several limitations. First, it was designed as a narrative synthesis rather than a systematic review or meta-analysis; therefore, study identification was targeted and iterative, and the selected evidence map should not be considered exhaustive. Second, the review was restricted to English-language literature and did not include a formal risk-of-bias assessment or certainty-of-evidence grading. Third, direct evidence from LMIC pediatric populations remains limited, and much of the proposed framework necessarily relies on transferability reasoning from studies conducted in high-income settings. Finally, heterogeneity in respiratory phenotype definitions, sampling sites, sequencing methods, and analytical pipelines limits direct comparison across studies.

In conclusion, pediatric upper-airway microbiota research is most useful for LMIC settings when it supports context-aware respiratory phenotyping rather than diagnostic labeling. Future work should combine phenotype-specific hypotheses, pragmatic low-biomass workflows, and locally relevant metadata for respiratory susceptibility, antibiotic stewardship, and vaccine-effect evaluation.

Conflict of interest statement

The authors declare that they have no known competing financial

interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The authors thank Can Tho University of Medicine and Pharmacy for academic support during the preparation of this manuscript. We also acknowledge colleagues in pediatrics, microbiology, and respiratory medicine whose discussions helped refine the clinical and methodological framing of this review.

Funding

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Ethics approval

Ethics approval was not required because this article is a narrative review of previously published literature. The study did not involve primary data collection from human participants or animals, and no individual-level identifiable data were used.

Data availability

No new data were generated or analyzed for this article. All evidence discussed in this narrative review is available from the cited published sources.

Declaration of generative AI use

During the preparation of this work, the authors used ChatGPT to assist with English language editing, structural refinement, and formatting support. The authors reviewed and edited the content and take full responsibility for the integrity and accuracy of the final manuscript.

Authors' contributions

N.C.Q. contributed to conceptualization, methodology, literature search, data curation, visualization, writing – original draft, writing – review and editing, and project administration. N.T.D.T. contributed to the literature search, data curation, supervision, and writing – review and editing. T.D.H. contributed to methodology, validation,

supervision, and writing – review and editing. T.T.T. contributed to supervision and writing – review and editing. T.H.H.D. contributed to methodology, data curation, and writing – review and editing. All authors read and approved the final version of the manuscript and agree to be accountable for all aspects of the work.

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Edited by Lei Y, Pan Y, Zhang Q

Supplementary Table S1. Selected original studies directly informing respiratory phenotype-specific interpretation of pediatric nasal and nasopharyngeal microbiota patterns

Study	Country/setting	Design	Age group	Sampling site	Respiratory phenotype	Main microbiota finding	Why included
Bosch et al. (2017) [3]	Netherlands; population-based infant cohort	Prospective birth cohort with serial sampling	Infants from birth through early infancy	Nasopharyngeal samples	Respiratory infection burden / respiratory-health trajectory in early life	Distinct maturation trajectories of the infant respiratory microbiota were linked to later respiratory-health consequences; stable maturation patterns differed from infection-prone trajectories.	Supports the concept that early nasopharyngeal microbiota maturation is clinically relevant to respiratory-health trajectory, not merely colonization.
Biesbroek et al. (2014) [6]	Netherlands; pediatric cohort	Longitudinal cohort	Infants and young children	Nasopharyngeal samples	Respiratory health and bacterial succession	Early respiratory microbiota composition was associated with later bacterial succession patterns and respiratory health.	Foundational longitudinal evidence linking early nasopharyngeal community composition with respiratory-health outcomes.
Teo et al. (2015) [7]	Australia; Childhood Asthma Study cohort	Prospective birth cohort with repeated illness sampling	Infants during the first year of life	Nasopharyngeal aspirates	Virus-associated ARI, lower respiratory infection severity, and later asthma risk	Virus-associated ARI episodes showed transient expansion of Streptococcus, Moraxella, or Haemophilus; microbiota patterns were linked to lower-airway spread, inflammatory severity, and later asthma risk.	Key phenotype-rich cohort connecting nasopharyngeal pathobiont-rich profiles with acute infection severity and later asthma risk.
Teo et al. (2018) [10]	Australia; childhood respiratory disease cohort	Longitudinal cohort	Children in early life	Nasopharyngeal samples	Wheezing, allergic sensitization, and asthma-related respiratory disease	Airway microbiota dynamics suggested a critical window in which pathogenic bacteria interacted with allergic sensitization in childhood respiratory disease.	Supports phenotype-specific interpretation for wheeze/asthma pathways rather than a simple taxonomic description.
Chonmaitree et al. (2017) [11]	United States; infant cohort	Prospective longitudinal cohort	Infants	Nasopharyngeal samples	Viral URTI and acute otitis media	Nasopharyngeal microbiota changed during viral URTI and AOM, involving otopathogen-related genera such as Streptococcus, Haemophilus, and Moraxella.	Directly supports the nasopharynx as a source niche for AOM during viral respiratory illness.
McCauley et al. (2021) [12]	United States; pediatric clinical cohort	Observational study	Children	Nasopharyngeal samples	Upper respiratory infection and sinusitis	Moraxella-dominated pediatric nasopharyngeal microbiota was associated with upper respiratory infection and sinusitis.	Adds phenotype-specific evidence for Moraxella-dominant communities in upper-airway respiratory disease.
Hasegawa et al. (2016) [13]	United States; multicenter bronchiolitis cohort	Multicenter prospective cohort	Infants hospitalized for bronchiolitis	Nasopharyngeal samples	Bronchiolitis severity	Haemophilus-dominant nasopharyngeal profiles were associated with more severe bronchiolitis phenotypes.	One of the strongest phenotype-specific studies linking bacterial community type with bronchiolitis severity.
Ederveen et al. (2018) [14]	Netherlands; hospitalized RSV cohort	Clinical cohort	Infants hospitalized with RSV infection	Nasopharyngeal samples	RSV infection severity and mucosal inflammation	Haemophilus was overrepresented in infants hospitalized with RSV and was associated with higher viral load and enhanced mucosal CXCL8 responses.	Provides phenotype-specific evidence for a Haemophilus-RSV-inflammation signal.
Mansbach et al. (2019) [15]	United States; RSV bronchiolitis cohort	Multicenter cohort with viral-clearance outcome	Infants hospitalized with bronchiolitis	Nasopharyngeal samples	RSV bronchiolitis and delayed viral clearance	Haemophilus-dominant nasopharyngeal microbiota was associated with delayed RSV clearance after adjustment for clinical factors.	Supports a clinically meaningful bacterial-viral interaction involving RSV clearance.
Penela-Sanchez et al. (2023) [16]	Spain; pediatric RV/EV LRTI setting	Case-control study	Children with rhinovirus/enterovirus lower respiratory tract infection	Nasopharyngeal samples	Rhinovirus/enterovirus LRTI severity	Children's nasopharyngeal microbiota composition varied by RV/EV infection severity; severe infection showed lower diversity and Haemophilus-predominant profiles, whereas Dolosigranulum-enriched profiles were related to respiratory health.	Strengthens LRTI evidence beyond RSV and links nasopharyngeal microbiota with viral LRTI severity.
Man et al. (2019) [17]	Netherlands; hospital and community case-control setting	Matched case-control study plus paired upper/lower airway substudy	Children with LRTI and matched healthy controls	Nasopharyngeal swabs; paired endotracheal aspirates in severe LRTI substudy	Lower respiratory tract infection	Nasopharyngeal microbiota, viral microbiota, and host characteristics distinguished LRTI cases from healthy controls; paired sampling suggested nasopharyngeal microbiota can serve as a proxy for lower-airway microbiota in childhood LRTI.	Key LRTI study supporting upper-lower airway ecological linkage and the value of combining microbiota with host/viral information.
de Koff et al. (2021) [18]	Netherlands; childhood LRTI follow-up cohort	Prospective cohort with follow-up	Children with LRTI	Nasopharyngeal samples	LRTI and early recurrence of respiratory symptoms	Distinct nasopharyngeal microbiota during LRTI, including higher Haemophilus and lower Corynebacterium/Dolosigranulum, was related to early recurrence of respiratory symptoms and diminished microbiota recovery.	Adds longitudinal post-LRTI evidence showing that recovery dynamics may matter as much as acute illness profiles.

Study	Country/setting	Design	Age group	Sampling site	Respiratory phenotype	Main microbiota finding	Why included
Sakwinska et al. (2014) [19]	Switzerland; pediatric pneumonia and control setting	Case-control / comparative study	Children with pneumonia and healthy children	Nasopharyngeal samples	Pneumonia and pneumonia etiology	Nasopharyngeal microbiota differed between healthy children and pneumonia patients; viral pneumonia was associated with <i>Moraxella lacunata</i> , whereas nonviral pneumonia showed higher abundance of typical bacterial-pathogen OTUs.	Foundational sequencing-based study directly comparing nasopharyngeal microbiota in pediatric pneumonia and health.
Pettigrew et al. (2016) [20]	United States; hospitalized CAP cohort	Prospective clinical cohort	Children 6 months to <18 years hospitalized with CAP	Nasopharyngeal/oropharyngeal samples plus induced sputum	Community-acquired pneumonia severity	Microbiota profiles differed between induced sputum and NP/OP samples. Sputum, but not NP/OP, microbiota profiles were associated with CAP severity outcomes.	Important cautionary CAP study showing that upper-airway samples may not always capture severity-associated lower-airway signals.
Claassen-Weitz et al. (2025) [21]	South Africa; Drakenstein Child Health Study	Nested case-control study within a birth cohort	Infants during the first year of life	Nasopharyngeal swabs	Infant lower respiratory tract infection	<i>Haemophilus</i> , <i>Klebsiella pneumoniae</i> , high-density <i>Streptococcus pneumoniae</i> , and several viruses were associated with LRTI; <i>Dolosigranulum</i> and <i>Corynebacterium</i> were reduced in LRTI cases.	Major LMIC birth-cohort evidence linking nasopharyngeal bacteria, viruses, and LRTI risk with adjustment for age and antibiotic therapy.
Makori et al. (2026) [22]	Kenya and Uganda; multicenter severe pneumonia cohort	Cross-sectional microbiome study nested within a randomized trial	Children hospitalized with severe pneumonia	Nasopharyngeal swabs	Severe pneumonia and clinical outcomes	Nasopharyngeal microbiome composition was characterized in children hospitalized with severe pneumonia in East Africa and explored in relation to clinical features, viral testing, comorbidities, and outcomes.	Direct LMIC severe-pneumonia evidence; important for transferability because it integrates nasopharyngeal microbiota with rich clinical metadata.
Lu et al. (2017) [23]	China; pediatric pneumonia setting	Comparative study	Children with MPP and non-MPP plus healthy controls	Nasopharyngeal and oropharyngeal samples	<i>Mycoplasma pneumoniae</i> pneumonia versus non-MPP	NP and OP microbiota differed between pneumonia patients and healthy children; diseased children had lower diversity and simpler co-occurrence networks, with NP-OP similarity increasing during pneumonia.	Supports the relevance of nasopharyngeal and oropharyngeal microbiota patterns in pediatric pneumonia phenotyping.
Dai et al. (2018) [24]	China; <i>Mycoplasma pneumoniae</i> pneumonia cohort	Comparative microbiota study	Children with <i>Mycoplasma pneumoniae</i> pneumonia	Upper- and lower-respiratory samples including nasopharyngeal/oropharyngeal components	<i>Mycoplasma pneumoniae</i> pneumonia	Upper- and lower-respiratory microbiota showed concordance patterns in children with <i>Mycoplasma pneumoniae</i> pneumonia.	Extends pneumonia evidence to upper-lower airway concordance, while retaining relevance to nasopharyngeal/oropharyngeal interpretation.
Henares et al. (2021) [25]	Spain; pediatric disease-comparison cohort	Case-control / comparative study	Children	Nasopharyngeal samples	Respiratory health status, viral URTI, and invasive pneumococcal disease	Nasopharyngeal microbiota differed by respiratory-health status; invasive pneumococcal disease showed pathogen enrichment and depletion of potentially protective taxa.	Supports phenotype-specific interpretation across healthy, viral respiratory illness, and severe bacterial disease states.
Camelo-Castillo et al. (2019) [26]	Spain; invasive pneumococcal disease setting	Case-control study	Children	Nasopharyngeal samples	Invasive pneumococcal disease	Children with invasive pneumococcal disease showed nasopharyngeal bacteria with potential disease-promoting and protective effects.	Supports the idea that severe pneumococcal phenotype reflects both pathogen enrichment and loss of protective competitors.
Kelly et al. (2017) [27]	Botswana; pediatric respiratory infection setting	Observational clinical study	Children with respiratory infections	Nasopharyngeal samples	Pediatric respiratory infections in an African setting	Nasopharyngeal microbiota profiles were described in children with respiratory infections in Botswana, providing original LMIC data on respiratory-infection-associated upper-airway communities.	Important LMIC-derived evidence; retained as contextual phenotype evidence, although detailed phenotype-specific comparisons were more limited than in some HIC cohorts.
Salter et al. (2017) [28]	Thailand/Myanmar border; South East Asian birth cohort	Longitudinal infant cohort	Infants during the first year of life	Nasopharyngeal swabs	Illness episodes and pneumonia-related respiratory outcomes	Infant nasopharyngeal microbiota varied with age and illness; illness-related patterns were interpreted in relation to respiratory-health outcomes in a South East Asian cohort.	Provides regional low-resource evidence linking infant nasopharyngeal microbiota with illness rather than only early-life exposure.
Coleman et al. (2021) [29]	Australia; Aboriginal and Torres Strait Islander children	Observational study	Children aged 2-7 years	Nasal cavity swabs	Otitis media and nose health	Nasal microbiota differed in relation to otitis media status and nose health in Indigenous Australian children.	Retained because it provides nasal microbiota evidence for ear/nose disease in an underrepresented Indigenous child population; not classified as LMIC evidence.
Hou et al. (2022) [30]	Clinical pediatric asthma setting	Longitudinal / temporal dynamics study	Children with asthma exacerbation	Nasopharyngeal samples	Childhood asthma exacerbation	Temporal dynamics of the nasopharyngeal microbiome were related to childhood asthma exacerbation.	Adds asthma-specific evidence beyond infancy and supports temporal rather than single-time-point interpretation.
Wen et al. (2018) [31]	China; influenza A pediatric setting	Case-control / comparative study	Children with influenza A and healthy children	Nasopharyngeal and oropharyngeal samples	Influenza A infection	Children with influenza A had distinct nasopharyngeal and oropharyngeal microbiota compared with healthy children.	Supports phenotype-specific microbial differences in viral respiratory infection outside Western high-income cohorts.

Study	Country/setting	Design	Age group	Sampling site	Respiratory phenotype	Main microbiota finding	Why included
Teng et al. (2024) [32]	China; pediatric allergic rhinitis setting	Cross-sectional clinical study	Children with allergic rhinitis	Nasal samples	Allergic rhinitis and symptom severity	Nasal microbiome features, including diversity measures and selected taxa, correlated with allergic rhinitis symptom severity.	Original pediatric allergic-rhinitis evidence focused on nasal microbiota and symptom severity.
Tang et al. (2024) [33]	China; pediatric allergic rhinitis and environmental microbiome study	Case-control multi-site microbiome study	Children with physician-diagnosed allergic rhinitis and matched controls	Nasal swabs, with dermal/oral swabs and indoor-dust samples as contextual sites	Allergic rhinitis and environmental microbial exposure	Nasal microbiota differed in children with allergic rhinitis and was evaluated alongside dermal, oral, and indoor-dust microbial communities.	Retained because the nasal microbiota component directly informs pediatric allergic rhinitis; non-nasal sites are interpreted as contextual environmental exposure data.

Notes: This table presents a selected evidence map of original pediatric studies that directly linked nasal or nasopharyngeal microbiota patterns with respiratory-health phenotypes. It is not an exhaustive catalogue of all pediatric nasopharyngeal microbiome studies identified in previous reviews. Studies focused only on microbiota determinants, carriage, methodological development, or exposure effects without a respiratory-health phenotype were excluded from this phenotype-specific evidence map, although they were considered where relevant for contextual interpretation or methodological priorities.