Upper Respiratory Tract Colonization With *Streptococcus pneumoniae* in Adults

Authors: Adriano Arguedas,[a] Krzysztof Trzciński,[b] Katherine L. O'Brien,[c]* Daniela M. Ferreira,[d] Anne L. Wyllie,[e] Daniel Weinberger,[e] Leon Danon,[f] Stephen I. Pelton,[g] Chiara Azzari,[h] Laura L. Hammitt,[c] Raquel Sá-Leão,[i] Maria-Cristina C. Brandileone,[j] Samir Saha,[k] Jose Suaya,[l] Raul Isturiz,[a] Luis Jodar,[a] Bradford D. Gessner[a]

[a]Vaccines Medical Development & Scientific/Clinical Affairs, Pfizer Inc, Collegeville, PA, USA

[b]University Medical Centre Utrecht, Utrecht, Netherlands

[c]Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, USA

[d]Liverpool School of Tropical Medicine, Liverpool, UK

[e]Department of Epidemiology of Microbial Diseases, Yale School of Public Health, New Haven, CT, USA

[f]University of Exeter, Exeter, UK

[g]Boston Medical Center, Boston, MA, USA

[h]Meyer Children's Hospital and University of Florence, Florence, Italy

[i]Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa, Oeiras, Portugal

[j]Adolfo Lutz Institute, São Paulo, Brazil

[k]Bangladesh Institute of Child Health, Matuail, Dhaka, Bangladesh

[l]Vaccines Medical Development & Scientific/Clinical Affairs, Pfizer Inc, New York, NY, USA

[*]Present address: World Health Organization, Geneva, Switzerland

Corresponding Author:

Adriano Arguedas, MD
Pipeline Vaccine Lead for Emerging Markets
Vaccines Medical Development & Scientific/Clinical Affairs
Pfizer Inc
500 Arcola Road
Collegeville, PA 19426, USA
Email: Adriano.Arguedas@pfizer.com
Telephone number: (484) 865-5218

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Author contact information

Name	Job title	Postal address
Adriano Arguedas, MD	Pipeline Vaccine Lead for Emerging Markets	Pfizer, Inc., 500 Arcola Road, Collegeville, PA 19426
Krzysztof Trzciński, PhD	Associate Professor	UMC Utrecht Heidelberglaan 100 3584 CX Utrecht
Katherine L. O'Brien, MDCM, MPH, FRCPC	Director-Immunization, Vaccines and Biologicals Department	World Health Organization (WHO) Main Headquarters: Geneva, Switzerland
Daniela M. Ferreira, PhD	Professor of Respiratory Vaccines and Infection Immunology	Liverpool School of Tropical Medicines; Liverpool, United Kingdom
Anne L. Wyllie, PhD	Associate Research Scientist	Yale School of Public Health, New Haven, CT 06511
Daniel Weinberger, PhD	Associate Professor of Epidemiology	Yale School of Public Health New Haven, CT 06511
Leon Danon, MSci, MSc, PhD	Senior Lecturer in Data Analytics	University of Exeter, Exeter, United Kingdom
Stephen I. Pelton, MD	Professor of Pediatrics and Epidemiology	Boston University, Boston, MA, 02215
Chiara Azzari, MD, PhD	Pediatric Clinic II, Director	University of Florence Piazza San Marco, 4 - 50121 Firenze. Florence, Italy
Laura L. Hammitt, MD	Associate Professor	Johns Hopkins University, Baltimore, MD 21218
Raquel Sá-Leão, PhD	Associate Professor	Universidade Nova de Lisboa, Lisboa, Portugal
Maria-Cristina C. Brandileone, PhD	Secretary of Health for the State of São Paulo	Instituto Adolfo Lutz Av. Dr. Arnaldo, 355 - Pacaembu, São Paulo - SP, 01246-000, Brazil
Samir Saha, MS, PhD	Senior Consultant	Dhaka Sishu (Children's) Hopsital Sher-e-Bangla Nagar, Dhaka-1207, Bangladesh
Jose Suaya, MD, MPH, PhD	Senior Medical Director	Pfizer, Inc, 235 E.42nd Street, New York, NY 10017
Raul Isturiz, MD	Vice President and Head of North America Region	Pfizer, Inc., 500 Arcola Road, Collegeville, PA 19426
Luis Jodar, PhD	Chief Medical Officer	Pfizer, Inc., 500 Arcola Road, Collegeville, PA 19426
Bradford D. Gessner, MD, MPH	Global Medical Lead	Pfizer, Inc., 500 Arcola Road, Collegeville, PA 19426

ABSTRACT

Introduction: Most of the current evidence regarding pneumococcal upper respiratory colonization in adults suggests that despite high disease burden, carriage prevalence is low. Contemporary studies on adult pneumococcal colonization have largely followed the pediatric approach by which samples are obtained mostly from the nasopharynx and bacterial detection is evaluated by routine culture alone. Recent evidence suggests that the “pediatric approach” may be insufficient in adults and pneumococcal detection in this population may be improved by longitudinal studies that include samples from additional respiratory sites combined with more extensive laboratory testing.

Areas covered: In this article, relevant literature published in peer review journals on adult pneumococcal colonization, epidemiology, detection methods, and recommendations were reviewed.

Expert opinion: Respiratory carriage of *Streptococcus pneumoniae* has been underestimated in adults. Contemporary pneumococcal carriage studies in adults that collect samples from alternative respiratory sites such as the oropharynx, saliva, or nasal wash; are culture-enriched for pneumococcus; and use molecular diagnostic methods designed to target two pneumococcal DNA sequences should enhance pneumococcal detection in the adult respiratory tract. This finding may have implications for the interpretation of dynamics of pneumococcal transmission and vaccination.

Keywords: adults, carriage, colonization, pneumococcal, *Streptococcus pneumoniae,* upper respiratory tract

ARTICLE HIGHLIGHTS

- Most of the evidence on pneumococcal upper respiratory tract carriage, associated implications, transmission, and dynamics following vaccination with pneumococcal vaccines have been obtained from studies performed in children.

- In spite of the bimodal incidence of pneumococcal infections with peaks in children under 5 years and adults 65 years and older, conventional nasopharyngeal carriage studies in ≥65-year old adults have found low pneumococcal respiratory carriage (0%–6%).

- Most carriage studies performed in adults have followed the pediatric approach in which a single swab from the nasopharynx, sometimes obtained together with an oropharyngeal sample, was collected and processed following standard culture recommendations.

- Contemporary studies suggest that pneumococcal detection in the respiratory tract of adults has been underestimated and that pneumococcal detection in this age group is enhanced in longitudinal studies that obtain samples from various respiratory sites, such as nasal washes, oropharynx, and saliva and by the use of culture enrichment and quantitative PCR targeting two pneumococcal DNA sequences.

- Given the importance of understanding the dynamics of pneumococcal carriage in adults and the effect of pneumococcal conjugate vaccines on respiratory carriage, future studies in adults should implement additional molecular techniques to measure pneumococcal carriage in adults and the potential of adult-to-adult or adult-to-children pneumococcal transmission.

1. INTRODUCTION

A large proportion of pneumococcal disease burden is represented by non-bacteremic pneumonia in children and adults and otitis media in children, outcomes that are caused by extension of mucosal pneumococcal colonization [1-4]. Pneumonia is a major cause of mortality in children younger than 5 years, representing 16% of childhood deaths worldwide in 2016 [5,6]. The burden of community-acquired pneumonia (CAP) in adults is also substantial, especially in the elderly and those with comorbidities [7,8].

Many studies have attempted to estimate CAP etiology and burden in adults but have been limited by the sensitivity and specificity of currently available diagnostic tests [9]. Recently, however, the availability of pneumococcal serotype-specific urine antigen assays with high sensitivity and specificity has improved the detection of pneumococcal CAP in adults [10].

Pneumococci commonly colonize the nasopharynx of young children, who are considered the major contributors to population transmission [1,11]. Colonization, also known as carriage, is considered a prerequisite for pneumococcal disease [1,12]. All people likely become colonized by pneumococci multiple times during their life, and colonization episodes typically resolve over days to months [11-13]. Pneumococcal disease peaks occur in children younger than 5 years and adults 65 years and older [14]; however, reported carriage prevalence has been higher among children than adults [15-29], which may partially explain why pneumococcal carriage studies have been most widely conducted in children [11,30-33].

Despite the wealth of information about pneumococcal carriage in children, information

regarding adult pneumococcal carriage and adult-to-adult and adult-to-children transmission is limited [34-36]. This review focuses on the available evidence regarding the epidemiology of pneumococcal colonization in adults, the discrepancies in colonization rates between children and adults, the differences in sample collection and laboratory processing, and current methodological recommendations. The goal is to identify best practices for evaluating adult pneumococcal carriage and to ascertain research gaps that may guide future studies on this topic.

2. PNEUMOCOCCAL CARRIAGE IN THE PEDIATRIC RESPIRATORY TRACT: CURRENT UNDERSTANDING

2.1 Epidemiology

Most modern studies of pneumococcal respiratory tract carriage have been conducted in children [11-13,37-42]. Among unvaccinated children, the detected nasopharyngeal pneumococcal colonization prevalence varies but appears to be highest in infants and young children (depending on factors such as setting, geographic area, and colonization detection methods) and decreases with age [13,37,43,44]. In high-income countries, the mean age for pneumococcal acquisition is approximately 6 months, whereas in developing countries, it occurs as early as the first days of life to 3 months of age [11,25,45,46]. Differences in carriage burden and transmission, environmental conditions such as crowding, conditions that cause immune suppression such as measles, and pneumococcal immunization schedules and coverage [11,45,47-50] may contribute to this variability in the age of pneumococcal acquisition among young

children in developed versus developing economies.

Pediatric carriage studies have provided useful information on the point prevalence of pneumococcal colonization among different populations [13,42,43,51,52]; characteristics of pneumococcal colonization, including carriage estimates by age and risk factors [13,53,54]; interactions between pneumococci and viruses or other bacterial pathogens [30]; disease-causing serotypes likely to emerge after vaccination with pneumococcal conjugate vaccines (PCVs) [42,55-59]; and invasive properties among diverse serotypes [32,60]. Pediatric carriage studies also provided early recognition of emerging antimicrobial resistance among pneumococcal strains [42,52,61,62], and of the relationship between antimicrobial use and resistance [33]. Additionally, carriage studies in children have contributed to our understanding of the direct and indirect effects of PCVs among vaccinated children and their contacts [63-66], and have been used to evaluate vaccine effectiveness [11]. Colonization data, when combined with information about the capacity of different strains to cause disease, are also used to understand and monitor the impact of vaccination on invasive disease prevalence [67]. Finally, pneumococcal carriage surveillance data have been considered by some groups as potential endpoints for pneumococcal vaccine licensure in children [68,69].

2.2 Assessment Methodology

The World Health Organization (WHO) recommendations for detecting pneumococcal carriage in children call for the collection of a single nasopharyngeal swab **(Fig. 1A)** [70]. This recommendation is based on minimal improvements in sensitivity achieved with

additional sampling, coupled by potential discomfort for the child [70]. Oropharynx sampling in children is not advocated because the added yield is small, and sampling sensitivity of the nasopharynx alone may be >90% [70,71]. Given that new molecular technologies are increasingly being used [15,35,36], controlled studies comparing sampling sensitivity between the nasopharynx and alternative sites associated with less discomfort would be valuable.

Nasopharyngeal swabs are placed in a transport medium upon collection [70]. Skim milk-tryptone-glucose-glycerin (STGG) medium is most commonly used, allowing recovery of live pneumococci after long-term storage at ultra-low temperatures (ie, –70°C or lower). Commercial media are also used, of which Amies transport medium is an example. To be stored frozen in Amies, glycerol needs to be added to the sample [35]. For samples to be tested with molecular tests (ie, real-time PCR), universal transport medium–containing vials can be used.

Further considerations must be observed regarding methodologies for colony identification and pneumococcal serotyping. For pneumococcal detection, nasopharyngeal samples are cultured on blood agar supplemented with 5 µg/mL gentamicin, which suppresses non-streptococcal species growth [70]. Pneumococcal identification has typically used isolates displaying a classic phenotype (alpha-hemolytic colonies) that are optochin susceptible and bile soluble [70]. In most pediatric carriage studies [32,41,43,70], pneumococci are serotyped by the capsular reaction/swelling test (Quellung reaction or Neufeld test [Statens Serum Institut, Copenhagen, Denmark]), which is the current “gold standard” method recommended by the WHO [70]. This is in part because of the experience using this methodology as part of the PCV clinical

development program including carriage studies (Dr. Katherine O'Brien, unpublished data). However, this method is labor-intensive, expensive, and requires the selection of a single colony for characterization; therefore, it is poorly suited to detecting carriage of multiple serotypes unless they are phenotypically distinct on the culture plate [36,70,72]. DNA-based approaches have also been developed for detection and serotyping, including microarray, sequencing, and traditional or quantitative PCR (qPCR) [73-75]. These approaches have been demonstrated to be highly sensitive in previous studies [15,36,76], and potentially represent a viable method for sample detection in children. However, caution is needed in interpreting PCR-serotyping assays performed directly on clinical samples because of suspected non-pneumococcal homologs of genes previously thought to be serotype-specific pneumococcal genes [77,78].

In some protocols, samples are passed through an initial broth culture step for further enrichment, such as the enrichment broth developed at the US Centers for Disease Control and Prevention that increases sensitivity of pneumococci detection by either conventional culture on agar medium or by molecular methods (**Figure 1B**) [79]. Alternatively, swabs can be tested with real-time PCR [80-84], which is suitable for diagnosis and serotyping and can detect co-carriage of multiple serotypes when present [85]. However, result interpretation requires caution because of potential false-positive findings [86,87].

In summary, most contemporary investigations of pneumococcal respiratory tract carriage have been conducted in children [11-13,37-42]. In children, it is recommended that a single nasopharyngeal swab be collected for culture-based pneumococcal carriage detection and serotyping using the capsular reaction/swelling test [70]; DNA-

based approaches with high sensitivity are also available for detection and serotyping [15,36,73-76] but limitations exist [77,78,86,87].

3. PNEUMOCOCCAL COLONIZATION IN THE ADULT RESPIRATORY TRACT: CURRENT UNDERSTANDING

3.1 Divergent Epidemiology in Children and Adults

Although older adults, particularly those aged ≥65 years, are at high risk of pneumococcal disease [16,88,89], pneumococcal respiratory carriage in adults has not been well characterized [24,81]. Moreover, despite disease peaks in young children and older adults, substantial differences in pneumococcal colonization prevalence between these populations are reported when comparable detection methods are used [36]. Detection of pneumococcal colonization with nasopharyngeal cultures is high among pediatric populations [11,53], but infrequent among ≥65-year old adults (0%–6%) [18-20,22,26,29,90-95] (**Table 1**). A number of potential explanations for the discrepancies in pneumococcal carriage prevalence between children and adults have been suggested. One relates to the maturation of the innate immune system with age and the development of serotype-specific immunity from multiple exposures during the first years of life resulting in short duration colonization episodes [16,19,96,97]. Additionally, studies have shown that adults have reduced pneumococcal receptors in the nasopharynx [16,19]. The combination of these characteristics may lower the pneumococcal density in the respiratory tract of adults compared with that of children or

may change the preferred ecological niche from the nasopharynx in children to the oral cavity in adults **(Figure 2)**.

3.2 Assessment Methodology

The conundrum of differing carriage prevalence between children and older adults is compounded by a relative paucity of contemporary data evaluating best methods and outcomes to evaluate pneumococcal carriage in adults. Determining the most accurate and biologically relevant means of measuring pneumococcal colonization in the adult respiratory tract is essential. For example, if colonization prevalence is incorrectly characterized as low in a population with high disease incidence because of insensitive methods, it may be erroneously concluded that enhanced invasiveness of certain serotypes exists in this population [98]. Alternatively, if low density colonization with subdominant strains is not biologically relevant for disease epidemiology, detecting these strains with highly sensitive assays may lead to incorrect conclusions about inherent strain characteristics.

Early studies measuring pneumococcal carriage in healthy adults collected only oral samples (saliva, throat swabs, or washes) and used mouse inoculation animal models [99-104]. These studies reported pneumococcal carriage prevalence values of 21% to 94% across all ages (**Figure 3**) [16,99-104], suggesting that current adult pneumococcal carriage prevalence may be underestimated if only nasopharyngeal cultures are analyzed.

Furthermore, recent studies suggest that adult pneumococcal carriage is more common than estimated previously and that the underestimation results from the use of

insensitive diagnostic tools (eg, limiting sampling to the nasopharynx, testing only by culture) [15,35,36,97]. These contemporary studies, described in detail below, used samples obtained from various sites, such as the nasopharynx, oropharynx, and saliva, and used molecular-based methods targeting specific conserved sequences of pneumococcal DNA [35,97].

3.3 Sampling Location for Adult Pneumococcal Carriage: Nasopharynx, Oropharynx, or Saliva

The optimal respiratory site for collection and identification of adult pneumococcal carriage is less clear than in children. The WHO updated their recommendations in 2013 accordingly, advising to include collection of both nasopharyngeal and oropharyngeal samples from adults, with prioritization of nasopharyngeal samples if sampling from only a single site is possible [70]. Nasal wash and oral cavity sample collection are better tolerated by participants, and some studies report oropharyngeal and saliva samples to be more informative for pneumococcal detection than nasopharyngeal samples in adults [15,16,35,36,70,105,106].

The WHO recommendation does not include collection of saliva samples or use of molecular-based diagnostic methods for adult samples, and the addition of oropharyngeal samples for culture only have not improved the detection of pneumococci in samples from adults [27,28,34-36].

Although saliva samples obtained from healthy adults and inoculated in the animal model were successfully used in early pneumococcal surveillance studies [99-104], ethical limitations exist with this approach. Currently, the main difficulty with the use of

oral samples for pneumococcal detection is their polymicrobial nature, being rich in respiratory aerobic and anaerobic bacteria, including pathogenic and nonpathogenic species of *Haemophilus* sp, *Neisseria* sp, and *Staphylococcus* sp, and various alpha hemolytic non-pneumococcal streptococci [16,107,108]. This complicates identification and isolation of pneumococci, with some streptococcal species producing atypical results in classical diagnostic assays; therefore, the use of multiple subcultures on selective medium or molecular diagnostic methods are required [16,76,78,87,107-110].

3.4 Molecular Techniques for Pneumococcal Identification in the Respiratory Tract of Adults

Because of the suspicion that culture-based pneumococcal detection methods are insensitive in adults, recent surveillance studies have evaluated the use of molecular-based detection technologies that target specific bacterial DNA sequences [15,29,35,36,71,85,111]. Several PCR-based diagnostic tests for pneumococcal identification have been proposed [15,78,108,112-115], but their usefulness has been hampered by a lack of specificity and sensitivity. Currently, the most promising approaches are real-time PCR–based assays targeting specific sequences of *lytA* (the major autolysin) in combination with the detection of SP2020 (a putative transcriptional regulator) or *piaB* (a permease gene of the pia ABC transporter) [15,76,78,116].

One of the benefits of molecular-based methods is that, unlike the Quellung test, they do not require a pure isolate and can be performed directly on human fluid such as respiratory samples and can detect the presence of multiple serotypes. They also may be useful in cases in which antimicrobial treatment has started [116-118]. Real-time

PCR (or qPCR) has greater sensitivity than multiplex sequential PCR in serotyping pneumococci [85,87,116].

A major concern with molecular diagnostic tests for identifying pneumococci among respiratory samples is the possibility of nonspecific or misleading results. False positive signals may occur because of the presence of non-pneumococcal streptococci carrying homologous pneumococcal genes, particularly in oral samples, which may produce misleading signals [78,86,87,119]. Additionally, the detection of pneumococcal DNA in saliva does not necessarily indicate that bacteria are viable or important contributors to disease risk [36,87]. Additional studies are necessary to further support the specificity of molecular diagnosis for the detection of adult pneumococcal carriage and to show the potential for positive saliva samples to contribute to transmission [120]. Molecular methods currently used for pneumococcal detection in the respiratory tract are more sensitive and specific than those previously used and involve qPCR testing for specific genes within culture-enriched samples and use of stringent definitions [15,35,36,76]. PCR testing has the disadvantage of potential non-specificity [121], with detection of pneumococcal DNA even if this reflects a situation with little clinical or biological consequences (eg, density so low that transmission does not occur or having genetic material carried by non-pneumococcal Streptococci). As assay sensitivity may be pushing the limits of our interpretive understanding, it is critical that studies address this issue.

In summary, the optimal respiratory site for detection of pneumococcal carriage in adults is not well elucidated and molecular-based detection methods are being developed to improve sensitivity and to define additional outcomes such as density; these approaches

have both benefits and limitations [15,16,35,36,70,78,105,106,108,112-115]. Described below and summarized in **Table 2** are studies in adults comparing different sampling sites and detection assays and assay refinement methods for assessing pneumococcal colonization.

4. STUDIES ASSESSING METHODOLOGIES USED IN CARRIAGE STUDIES IN ADULTS

4.1 Assessment of Sampling Sites and Detection Methods

To evaluate various respiratory niches, a cross-sectional study conducted in Israel examined the importance of nasopharyngeal and oropharyngeal samples for the detection of pneumococci in 216 children 5 years and younger and their mothers [71]. A single sample of each type was obtained from each participant. Respiratory samples were inoculated onto Columbia agar plates with 5% sheep blood and 5 µg/mL gentamicin; bacterial identification followed standard recommendations. Nasopharyngeal samples alone would have missed only 2% of overall pneumococcal isolates cultured in children but 42% of overall pneumococcal isolates cultured in mothers, while oropharyngeal cultures alone would have missed 73% of pneumococcal isolates in children and 45% of maternal isolates. These findings suggest that nasopharyngeal cultures alone are sufficient in children, whereas both nasopharyngeal and oropharyngeal cultures are needed in parent-aged adults to optimize pneumococcal colonization detection.

Two recent studies found a low pneumococcal colonization prevalence among adults 65 years and older, highlighting the complexity of pneumococcal colonization studies in adults (**Table 1**) [28,29,81,122]. The first was a multicenter surveillance assessment of prevalence and serotype distribution of pneumococcal carriage in individuals 65 years and older at centers in four US states [28,81]. Samples (1 each) from the nasopharynx and oropharynx were collected for routine culture. Along with conventional culture-based diagnostic screening, all samples underwent molecular testing using qPCR targeting *lytA*, as described previously [113]. Among 2989 samples, pneumococcal carriage prevalence was 1.8%, with 1.5% prevalence based on culture-positive samples from the nasopharynx or oropharynx and 0.3% *lytA*-positive samples from nasopharyngeal samples [81]. These findings are similar to the prevalence of adult pneumococcal colonization reported from other studies using nasopharyngeal samples [16,19,22,35]. However, important limitations of this study were that qPCR testing was only performed among nasopharyngeal samples, and saliva samples were not obtained thereby precluding definitive characterization of the pneumococcal colonization prevalence in this population.

The second study was a cross-sectional, prospective assessment that enrolled participants of all ages from the Navajo Nation and White Mountain Apache Tribal lands in the southwest United States between October 2015 and November 2017 [29,122]. These communities have historically high prevalence of pneumococcal colonization in children and pneumococcal infections in children and adults [29,123,124]. Nasopharyngeal swabs for culture were collected at all ages, and a sample from the oropharynx was additionally collected from adults [29,122]. Both samples were collected

using flocked swabs, inoculated into STGG, and cultured initially in broth enrichment media [29]. Nasopharyngeal carriage prevalence by culture was 49.5% (297/600) in children younger than 5 years, 8.9% (53/597) in adults 18 to 64 years of age, and 6.0% (18/299) in adults 65 years and older. Similar to the previous study, limitations of this study are that saliva samples were not collected from adults, and qPCR results were absent. However, the authors of this study suggested the need for supplementary studies to determine the optimal sampling site and diagnostic assay [29].

Additionally, a recently published cross-sectional study evaluated the differences between culture-based and molecular methods in detecting pneumococcal nasopharyngeal colonization and the effects of age and colonization density on detection in healthy individuals [125]. Nasopharyngeal specimens were assessed from 982 healthy individuals (median age: 18.7 years) from 2010 to 2012 on the Navajo Nation and White Mountain Apache Tribal lands in the United States. Overall, samples from 35% of participants underwent broth-enrichment culture and 60% of samples were qPCR positive for *lytA*, with a 71% agreement between the two diagnostic tests. Interestingly, *S pneumoniae* was detected more frequently among samples with higher bacterial density while qPCR improved pneumococcal detection among adults 18 years and older with lower pneumococcal density [125].

Results from a longitudinal surveillance study designed to document cumulative incidence of pneumococcal colonization in adults 65 years and older were recently published [97]. One hundred community-dwelling adults from Rochester, New York, were recruited in 2015 and followed up for 12 months. Nasopharyngeal and oropharyngeal samples were obtained bimonthly. DNA was extracted from broth-

enhanced medium and tested in real-time PCR amplifying *lytA*. Colonization was defined as at least one oropharyngeal or nasopharyngeal sample positive for *lytA* via PCR as described previously [113]. PCR-positive samples were subcultured on gentamicin blood agar plates to identify and serotype pneumococci [97]. During the 12-month surveillance period, 57 colonization events among 41 participants were observed, resulting in a 12-month cumulative colonization prevalence of 41% by PCR and 14% by culture. Among 149 *lytA*-positive samples, viable pneumococci were isolated in 11% of samples, with pneumococcal-like isolates grown from oropharyngeal (56.4%), nasopharyngeal (25%), or from combined broth-enhanced samples (18.6%) [97]. The possibility of detecting false signals from the respiratory tract has been reported previously [107]. Nevertheless, use of qPCR methods targeting at least two pneumococcus-unique sequences (eg, *lytA*, *piaB*) should diminish the possibility of false-positive results [15,35,36].

In consideration of the limitations observed in pneumococcal sampling and detection in adults described above, studies conducted in the Netherlands provide insights regarding methodologic considerations for assay refinement **(Figure 1C)**. The first, which was performed during the fall/winter seasons of 2010 and 2011, used paired nasopharyngeal and oropharyngeal samples from 268 parents of 24-month-old children [15]. Nasopharyngeal samples were obtained using standard flexible swabs, and oropharyngeal samples were collected using rigid swabs under direct observation of the pharynx. Samples were cultured on agar selective for streptococci and harvests of all colony growth from these plates were considered to represent samples culture-enricheded for pneumococci. Extracted DNA were tested with qPCR for sequences

unique for *lytA* and *piaB* and considered positive for pneumococci when both targets were detected. While routine culture was as sensitive as molecular methods in detecting pneumococci in adult nasopharyngeal samples, routine culture sensitivity was low when oropharyngeal samples were tested. The number of oropharyngeal samples identified as positive for pneumococci dramatically increased when culture-enriched samples were analyzed with molecular methods or revisited with a second culture stage after positivity had been determined by qPCR. Additionally, oropharyngeal samples were superior to nasopharyngeal samples in pneumococcal carriage detection whether tested with culture or molecular methods. Findings for this age group were supported in a second study, which applied similar methods as well as saliva sampling, the latter of which resulted in superior molecular surveillance compared with nasopharyngeal sampling [126].

The authors reached several conclusions [15]. Underreporting of pneumococcal respiratory tract colonization in adults can occur if only routine nasopharyngeal or oropharyngeal cultures are obtained. Oropharyngeal was superior to nasopharyngeal sampling for detecting pneumococcal colonization when tested by qPCR. The use of qPCR testing can identify samples that produce viable pneumococcal isolates through further laboratory processing and with strict controls in place. Thus, this method may achieve higher sensitivity and specificity for the detection of pneumococci in the respiratory tract of adults and children.

Another study from the same group compared pneumococcal colonization prevalence among respiratory sites using different detection methods in older adults [35]. Samples from the nasopharynx, oropharynx, and saliva (270 per sample type) were collected

during the autumn/winter of 2011/2012 from 135 persons 60 to 89 years of age at the onset of influenza-like illness and 7 to 9 weeks later, after recovery from influenza-like illness. Nasopharyngeal samples were collected and processed as noted for the previous studies [15,36], and saliva samples were collected using a saliva collection system. qPCR was used to detect the *lytA* and *piaB* pneumococcal genes. Samples were considered positive for pneumococci when both genes were detected. Overall detection of pneumococcal carriage was higher via the molecular method (34%) versus culture (6%). Of note and as described above, by revisiting samples testing positive for pneumococci by the molecular method with additional culture steps, the number of culture-positive samples increased. Additionally, the number of saliva samples classified as pneumococcal-positive by the molecular method increased significantly by testing culture-enriched samples compared with uncultured saliva samples (ie, raw, direct testing). As in other studies [15,107], these data support the use of an enrichment step and suggest that viable pneumococci, rather than solely pneumococcal DNA, are the source of the signal. When considered together, these two studies highlight the importance of vigilance and using proper methods for detecting pneumococci in adults [15,35,126].

4.2 Experimental Human Pneumococcal Carriage Model

The experimental human pneumococcal carriage (EHPC) model was recently developed, allowing for fast, safe, and accurate analysis of the interaction between pneumococcal serotypes 6B, 15B and 23F in the upper respiratory tract and host [127] (and Dr. Daniela Ferreira, unpublished data from 14th European Meeting on the Molecular Biology of the Pneumococcus). This model has been tested in at least 1000

healthy adult volunteers (Dr. Daniela Ferreira, unpublished data), including vaccine efficacy studies, and is being studied in patients with mild, well-controlled asthma [127-129].

The EHPC is an important platform to understand microbiologic and mucosal properties associated with pneumococcal colonization in the respiratory tract. Volunteers are nasal inoculated with a pneumococcal strain diluted in saline at a determinate bacterial concentration [127]. Samples of various respiratory tract sites are then obtained, allowing analysis of host and bacterial properties associated with pneumococcal colonization, longitudinal follow-up of participants, and assessment of pneumococcal vaccine effects in preventing colonization.

Approximately 10% to 60% of volunteers become colonized following nasal inoculation within the EHPC protocol; this percentage varies depending on the serotype used (Dr. Daniela Ferreira, unpublished data from 14th European Meeting on the Molecular Biology of the Pneumococcus). The carriage prevalence is approximately 60% for the serotype 6B model, approximately 31% for serotype 15B, and approximately 10% to 16% for serotype 23F. The model has also estimated that after serotype 6B colonization is achieved, it remains detectable for approximately 22 days (Dr. Daniela Ferreira, unpublished data).

Data from the EHPC studies indicate that after nasal inoculation, respiratory colonization can take up to 24 hours to become established [130]. Individuals successfully colonized after nasal inoculation with serotype 6B lacked cytokine and neutrophil responses during the first hours and days following exposure, and bacterial

DNA was not found during the initial hours in saliva. Eight hours after nasal inoculation, pneumococcal density starts to decline in the anterior nares, suggesting the occurrence of pneumococcal migration toward the nasopharyngeal site, strong epithelial binding, or internalization of the pneumococci.

Among volunteers in whom nasal inoculation failed to establish respiratory colonization, the EHPC model identified two distinct pneumococcal clearance profiles [130]. The first group consisted of nasal clearers, among whom an immediate local bacterial clearance occurred and was associated with a strong baseline neutrophil activation and lack of proinflammatory response or bacterial clearance to the saliva. For these individuals, the model suggested that neutrophils play an important role in the prevention of pneumococcal colonization. A second path for pneumococcal clearance was observed in a group of individuals for whom pneumococci quickly reached the saliva, most likely because of effective nasal mucociliary activity (saliva clearance). Saliva clearers induced a strong proinflammatory response in the first day after exposure associated with concurrent induction of neutrophil responses.

The EHPC model was used to analyze the impact of 13-valent PCV (PCV13) on pneumococcal colonization in the respiratory tract of 100 healthy participants 18 to 50 years of age [128]. Participants in this double-blind placebo-controlled trial were randomly assigned to receive PCV13 (n=49) or hepatitis A vaccine (HAV; n=50) followed by inoculation with 80,000 CFU/100 mL of pneumococcal serotype 6B. Participants were followed for 21 days to determine pneumococcal colonization by routine culture of nasal wash. The PCV13 group had an overall significantly reduced 6B colonization prevalence (10%) compared with the HAV group (48%; $P<0.001$). At 3

weeks postvaccination, the serotype 6B colonization prevalence was 4.3% in the PCV13 group and 33.3% in the HAV group. Density of colonization and the area under the curve (density vs day) were reduced in the PCV13 compared with the HAV group following inoculation.

5. CONCLUSION

Adults 50 years and older, especially the elderly (ie, 65 years and older), represent a particularly vulnerable population for pneumococcal disease. Despite this, there is a relative dearth of information regarding pneumococcal respiratory colonization and transmission in adults. Here, we reviewed the available evidence and highlighted the overall low detection rates of adult pneumococcal colonization using nasopharyngeal cultures, a striking contrast to pediatric populations where both disease incidence and colonization rates are high. However, contemporary studies suggest that detection of pneumococcal colonization in adults can be improved by incorporating additional respiratory sampling sites and sensitive culture- and molecular-based techniques. Future studies should consider implementing these methodologies to further elucidate adult pneumococcal colonization and transmission dynamics to better guide preventative and therapeutic interventions.

In conclusion, based on the evidence reviewed herein, samples from the nasopharynx, oropharynx, and saliva should be obtained from adults 65 years and older for detection of pneumococci by culture and qPCR; the use of qPCR should be implemented with caution and ideally should target at least two pneumococcal genes. Depending on the

study objectives, samples may need to be collected longitudinally at prespecified time points.

6. EXPERT OPINION

Pneumococcal infection can progress to serious and sometimes fatal illness, with children and older adults at greatest risk [37]. While most children are transiently colonized at a young age [1], the prevalence of carriage reported in adults has generally been substantially lower than in children [16]. The current paradigm for the transmission of pneumococci argues that transmission occurs from toddlers and young children to all age groups [1,11].

PCV immunization programs for pediatrics have been very effective in decreasing acquisition and carriage of most vaccine-type pneumococci among children and interrupting transmission to unvaccinated populations including adults [131]. Importantly, reduction in the transmission of pneumococci has led to large indirect declines of vaccine-serotype pneumococcal disease in both pediatric and adult populations [63-66]. Recent surveillance data have, however, shown region-specific trends toward increased prevalence of certain pneumococcal serotypes in adults, despite highly effective pediatric immunization programs [1,132]. For these reasons, the accurate assessment of pneumococcal colonization in adults, particularly in those older than 65 years, is essential for a clear understanding of the biological link and risk of colonization events for pneumococcal disease. In addition, data on carriage from adults may provide valuable information that can help guide preventative and therapeutic interventions.

Previous studies on pneumococcal colonization in adults have followed the pediatric approach, with sampling of the nasopharynx for routine cultures [18-20,26,133]. However, data suggest that methods to assess pneumococcal colonization in adults should differ from those used in children [15,35,36,97]. Establishing robust practices for sampling to investigate pneumococcal colonization in adults is more complex than in children [70].

Further studies assessing the sensitivity and specificity of testing various respiratory niches, and that evaluate different diagnostic modalities, are needed to establish recommendations that are evidence based, including systematic evaluation of emerging data and the development of standards for colonization studies in adults. Initial studies should include sampling of all respiratory sites—such as the nasopharynx, oropharynx, saliva, and nasal washes—for *S pneumoniae* detection with standard cultures and using molecular diagnostic methods.

Overall, we think that pneumococcal carriage in adults has largely been underestimated to date. However, we speculate that the findings discussed here highlight key limitations and approaches to pneumococcal carriage studies in adults and will thereby steer future studies towards best practices in understanding pneumococcal transmission and vaccination dynamics.

During the next 5 years, we expect that implementing the collection and laboratory assessments discussed in this review will demonstrate higher colonization rates in adults, with some degree of adult-to-adult and adult-to-childhood pneumococcal transmission that might be prevented with the use of conjugated pneumococcal

vaccines in the population of older adults. This better understanding of adult pneumococcal colonization and transmission is key for guiding preventative and therapeutic interventions to impact the burden of pneumococcal disease in adults.

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AUTHOR CONTRIBUTIONS

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This study demonstrated the beneficial effect of PCV13 in the human experimental challenge model inoculated with *S pneumoniae* serotype 6B; compared with a control group vaccinated against hepatitis A, PCV13 recipients showed greater protection against pneumococcal colonization and a significantly lower bacterial density in those participants who were colonized.

Table 1. Pneumococcal Colonization of the Upper Respiratory Tract of Healthy Adults Identified by Cultures Only

Study	Population (Year)	Age	Number of Participants	Sample Source	Pneumococcal Colonization, %
Adler et al **[90]**	Community (2010–2017)	≥18 y	795	NP	6.5
Hammitt et al **[91]**	Community (2009–2017)	≥18 y	1978	NP	21.7
Becker-Dreps et al **[26]**	Retired community (2013–2014)	≥65 y	210	NP	1.9
Grant et al **[92]**	Community (2010–2012)	≥18 y	6628	NP	13
Sutcliffe et al **[93]**	Community (2010–2012)	≥18 y	513	NP	8–12
Roca et al **[94]**	Community (2010)	≥15 y	398	NP	17.6
Regev-Yochay et al [20]	Parents of children aged ≤5.5 y	Adults	376	NP	8

	(2009)				
Palmu et al [22]	Community (2003–2004)	≥65 y	590	NP	0.0–5.9
Abdullahi et al **[95]**	Community (2004)	≥50 y	107	NP	4.7
Regev-Yochay et al [19]	Community (2001)	Adults	1300	NP	3.7
Saravolatz et al [18]	Military (NR)	Adults	200	NP	1.5
Grant et al [29]	Community (2015–2017)	18–64 y	597	NP	8.9
		≥18 y	901	OP	0.6
		≥65 y	299	NP	6.0
Putnam et al [21]	Military (1994–1995)	Adults	915	OP	1.2
Milucky et al **[81]**	Community (2015–2016)	≥65 y	2989	NP, OP	1.8
Almeida et al [34]	Community (2010–2012)	>60 y	3361	NP, OP	2.3
Van Gils et al **[133]**	Parents of	Adults	953	NP, OP	24.4

	children aged 12 mo				
	Parents of children aged 24 mo (2005–2008)	Adults	926	NP, OP	19.9
Levine et al [24]	Military (2007)	Adults	742	NP, OP	10.8
Ridda et al [17]	Hospitalized elderly (2005–2006)	≥60 y	315	NP, OP	0
Millar et al [23]	Adults (1997–2000)	≥65 y	70	NP, OP	9

1061 NP=nasopharyngeal; NR=not reported; OP=oropharyngeal.

1062

Table 2. Pneumococcal Colonization of the Upper Respiratory Tract of Healthy Adults Identified by Cultures and/or qPCR

Study	Year	Country	Number of Participants	Age, y	Site	Analysis	Pneumococcal Colonization; n/N (%)
Krone et al [35]	2011–2012	Netherlands	135	≥60 y	NP	Culture	6/270 (2)
						qPCR	13/270 (5)
					OP	Culture	10/270 (4)
						qPCR	31/270 (11)
					Saliva	Culture	6/270 (2)
						qPCR	76/270 (28)
					NP, OP, and saliva	Culture	0/270 (0)
						qPCR	6/270 (2)
					NP, OP, or saliva	Culture	15/270 (6)
						qPCR	91/270 (34)
Martinelli et al 2016 **[132]**	2013–2015	Italy	195	≥65 y	NP	qPCR	76/195 (39)
Ansaldi et al 2013 **[135]**	2012	Italy	283	≥60 y	NP	qPCR	53/283 (19)
Suzuki et al 2006 **[115]**	NA	Japan	30	≥60 y	Saliva	qPCR	11/30 (37)
Trzciński et al 2013 [15]	2010–2011	Netherlands	268	Parents	NP	Culture	49/268 (18)
						qPCR	50/268 (19)

					OP	Culture	10/268 (4)
						qPCR	94/268 (35)
					NP and OP	Culture	7/268 (3)
						qPCR	39/268 (15)
					OP or NP	Culture	52/268 (19)
						qPCR	105/268 (39)
Hamaluba et al [37]	2010–2011	United Kingdom	100	Parents	NP	qPCR	9/100 (9.0)
			606	≥65 y	NP	qPCR	13/599 (2.2)
Becker-Dreps et al [26]	2010	United States	210	≥65 y	NP	qPCR	4/210 (1.9)
Almeida et al **[134]**	2015–2016	Portugal	87	25–50 y	NP, OP, and saliva	qPCR	25/87 (28.7)
Flamaing et al **[136]**	NA	Belgium	503	80 y (mean)	NP	qPCR	21/503 (4.2)
Esposito et al **[137]**	2015	Italy	417	All ages	OP	qPCR	41/417 (9.8)
			246	<75 y			28/246 (11.4
			171	≥75 y			13/171 (7.6)
van Duersen et al **[138]**	2007–2008	Netherlands	330	≥65 y	NP	Culture	16/330 (5)
						qPCR	32/330 (10)
					OP	Culture	16/330 (5)
						qPCR	58/330 (18)
					NP and OP	Culture	7/330 (2)
						qPCR	19/330 (6)

					NP or OP	Culture	25/330 (8)
						qPCR	71/330 (22)
Sutcliffe et al **[125]**	2010–2012	United States	63	>50	NP	Culture	18/63 (29)
						qPCR	24/63 (38)

NA=not available; NP=nasopharyngeal; OP=oropharyngeal; qPCR=quantitative PCR.

FIGURE LEGENDS

Figure 1. Standard diagnostic methods for the detection of pneumococci from the respiratory tract of children and adults. **A.** Pediatric method [70], **B.** Adult method without enrichment medium for pneumococcal growth and enhancement of DNA extraction used in the study by the CDC [81], and **C.** Adult method with samples culture-enriched for pneumococcal growth used in the Netherlands [15,35,87,126]. CDC=US Centers for Disease Control and Prevention; NP=nasopharyngeal; OP=oropharyngeal; qPCR=quantitative polymerase chain reaction; smPCR=single molecule polymerase chain reaction; STGG=skim milk-tryptone-glucose-glycerin. *Before placing on dry ice, saliva is supplemented with 10% (final concentration) glycerol; †all samples tested independent of *lytA* and *piaB* qPCR results; §samples positive for pneumococci when tested with molecular method.

Figure 2. Dynamics of pneumococcal colonization in the upper respiratory tract of children and adults [12,16,19,139,140]. qPCR=quantitative polymerase chain reaction.

Figure 3. Pneumococcal prevalence from the oral cavity of adults in early studies [16,99-104]. *Data are reported in Krone et al [16].

Fig. 1.

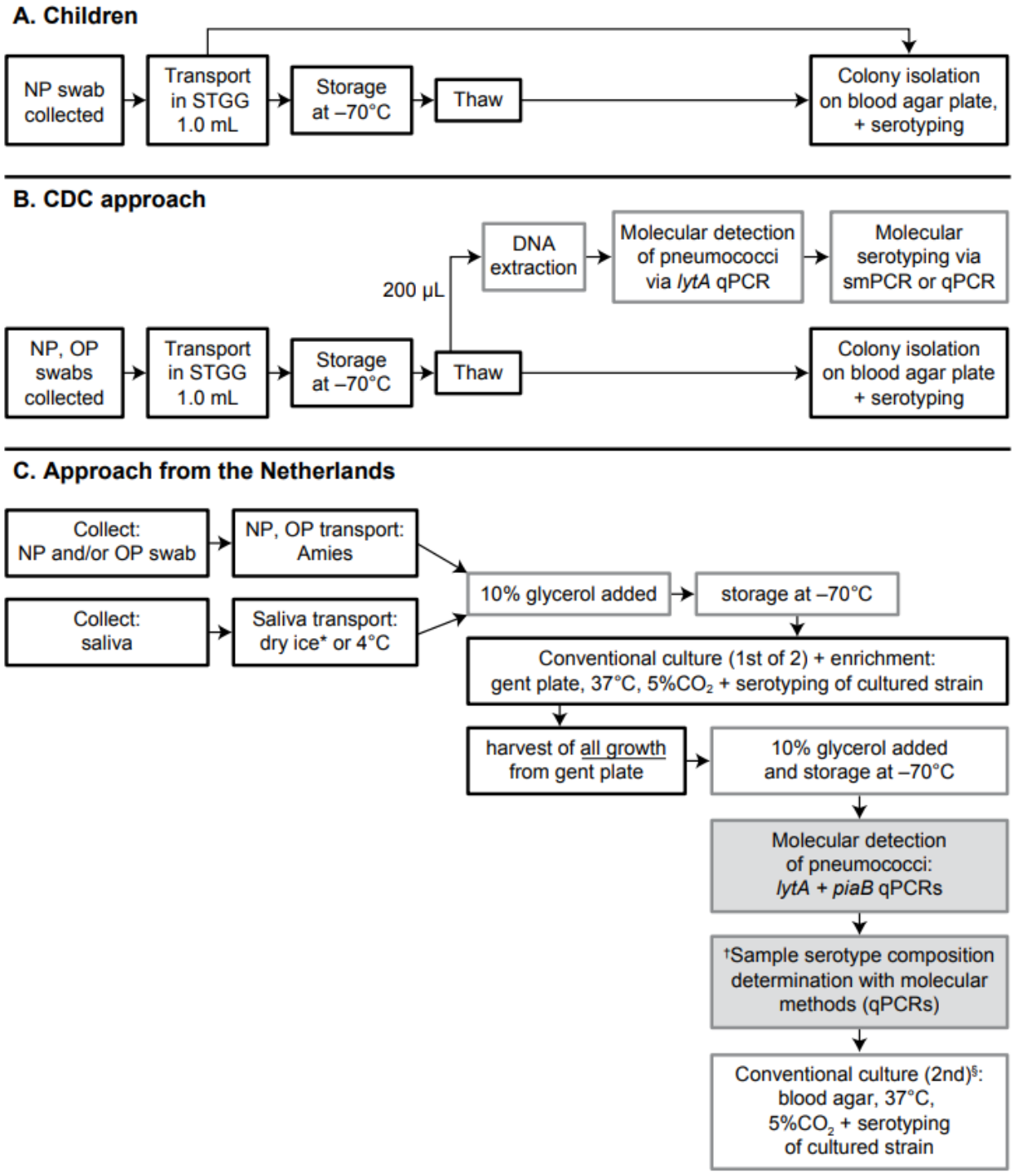

A. Children
NP swab collected
Transport in STGG 1.0 mL
Storage at –70°C
Thaw
Colony isolation on blood agar plate, + serotyping
B. CDC approach
200 µL
DNA extraction
Molecular detection of pneumococci via lytA qPCR
Molecular serotyping via smPCR or qPCR
NP, OP swabs collected
Transport in STGG 1.0 mL
Storage at –70°C
Thaw
Colony isolation on blood agar plate + serotyping
C. Approach from the Netherlands
Collect: NP and/or OP swab
NP, OP transport: Amies
Collect: saliva
Saliva transport: dry ice* or 4°C
10% glycerol added
storage at –70°C
Conventional culture (1st of 2) + enrichment: gent plate, 37°C, 5%CO2 + serotyping of cultured strain
harvest of all growth from gent plate
10% glycerol added and storage at –70°C
Molecular detection of pneumococci: lytA + piaB qPCRs
†Sample serotype composition determination with molecular methods (qPCRs)
Conventional culture (2nd)§: blood agar, 37°C, 5%CO2 + serotyping of cultured strain

Fig. 2.

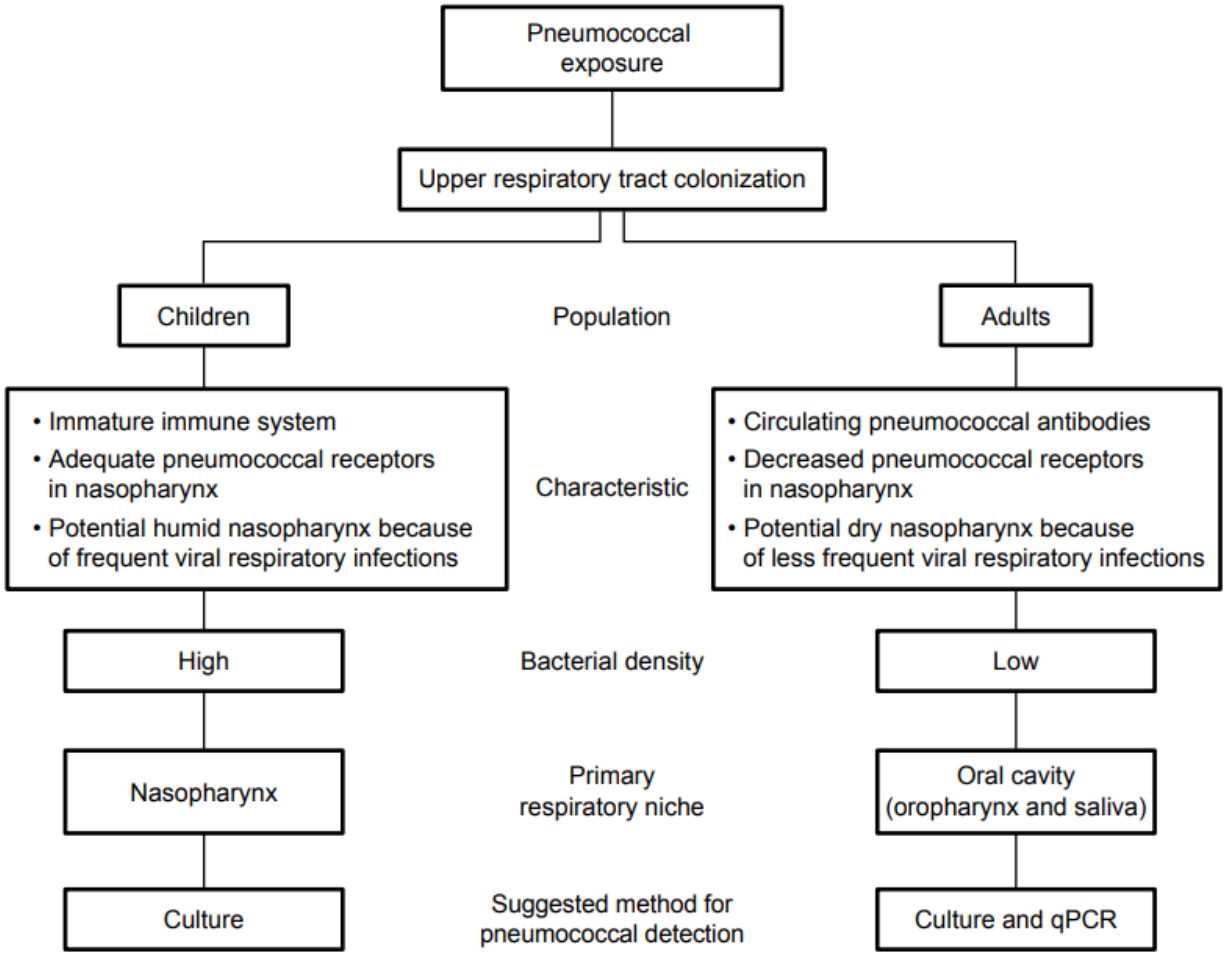
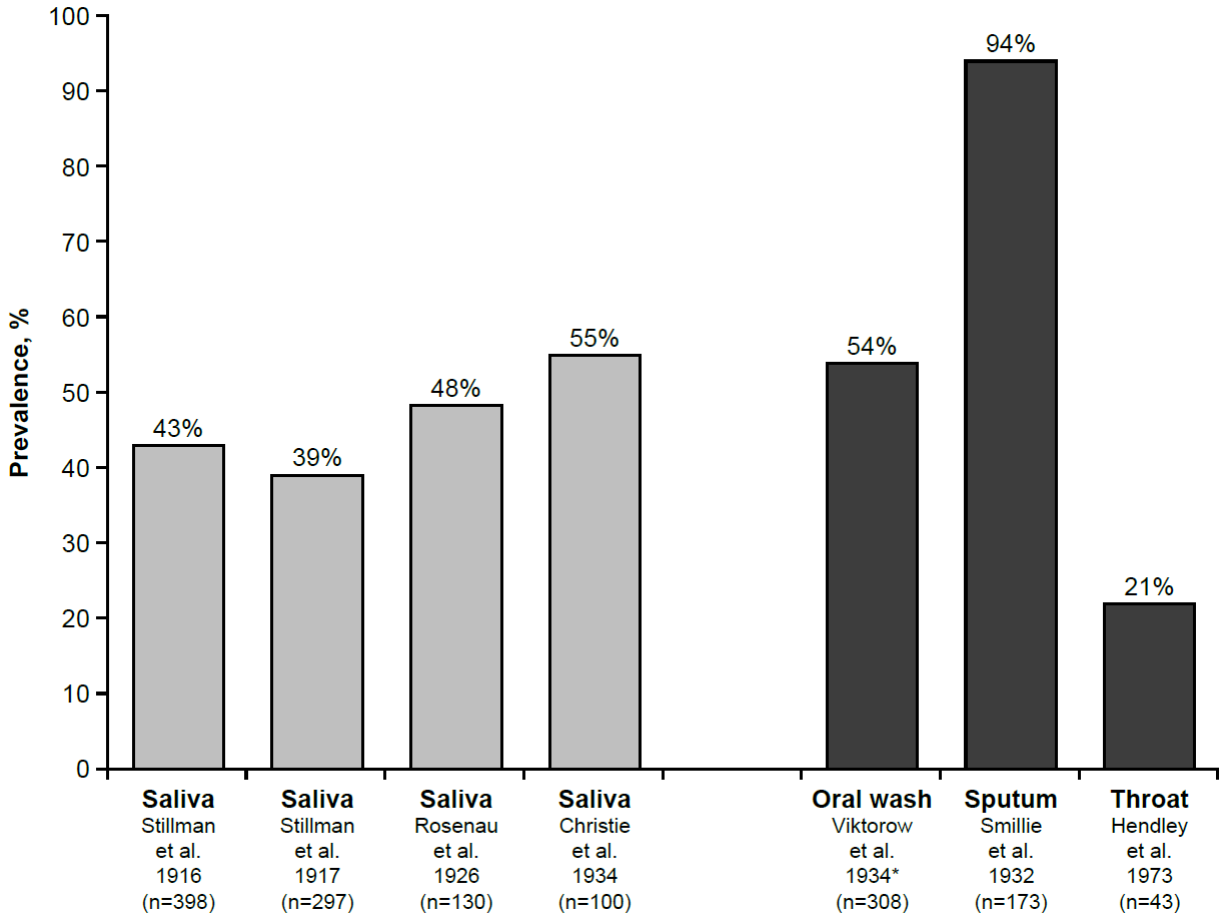

Pneumococcal exposure
Upper respiratory tract colonization
Children
Population
Adults
• Immature immune system
• Adequate pneumococcal receptors in nasopharynx
• Potential humid nasopharynx because of frequent viral respiratory infections
Characteristic
• Circulating pneumococcal antibodies
• Decreased pneumococcal receptors in nasopharynx
• Potential dry nasopharynx because of less frequent viral respiratory infections
High
Bacterial density
Low
Nasopharynx
Primary respiratory niche
Oral cavity (oropharynx and saliva)
Culture
Suggested method for pneumococcal detection
Culture and qPCR

Fig. 3.


Prevalence, %
100
90
80
70
60
50
40
30
20
10
0
43%
39%
48%
55%
54%
94%
21%
Saliva
Stillman
et al.
1916
(n=398)
Saliva
Stillman
et al.
1917
(n=297)
Saliva
Rosenau
et al.
1926
(n=130)
Saliva
Christie
et al.
1934
(n=100)
Oral wash
Viktorow
et al.
1934*
(n=308)
Sputum
Smillie
et al.
1932
(n=173)
Throat
Hendley
et al.
1973
(n=43)