

SYSTEMATIC REVIEW OPEN ACCESS

Prevalence, Antimicrobial Resistance, and Genetic Diversity of Non-Typeable *Streptococcus pneumoniae* Carriage: A Systematic Review and Meta-Analysis

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ABSTRACT

Background: Non-typeable *Streptococcus pneumoniae* (NT-Sp) strains lack detectable capsules and are often missed by traditional serotyping. While associated primarily with asymptomatic carriage, NT-Sp has emerged as a key player in antimicrobial resistance (AMR) gene dissemination and serotype replacement in the post-vaccine era. This systematic review and meta-analysis synthesize global evidence on NT-Sp carriage, its prevalence, antimicrobial resistance patterns, and genetic diversity.

Methods: A systematic search of PubMed, Scopus, Web of Science, Google Scholar, and ScienceDirect was conducted up to May 2025. Eligible studies reporting NT-Sp carriage, molecular typing, or AMR profiles were included. Data extraction and quality assessment followed PRISMA guidelines. A meta-analysis was performed to estimate pooled prevalence of NT-Sp carriage and resistance, with subgroup and meta-regression analyses exploring heterogeneity. Methodological quality was assessed using the Newcastle–Ottawa Scale adapted for cross-sectional studies. Only open-access and free full-text articles were included, which may have introduced selection bias.

Results: Forty studies from 23 countries were included. The pooled NT-Sp carriage prevalence was 4.64% (95% CI: 2.78%–6.92%), with substantial heterogeneity ($I^2 = 97.1\%$, 95% CI: 96.6%–97.5%). Subgroup analyses revealed geographic variation, with Oceania showing the highest prevalence (7.24%, 95% CI: 1.39%–17.04%) and North America the lowest (0.44%, 95% CI: 0.05%–1.11%). Age-stratified analysis showed that studies, including all ages had the highest estimate (29.88%, 95% CI: 0.00%–100.00%), while children had a pooled prevalence of 4.05% (95% CI: 0.00%–15.80%). Vaccination era did not significantly influence NT-Sp carriage ($p = 0.312$). Resistance was highest for co-trimoxazole (67.21%, 95% CI: 49.92%–82.73%), penicillin (44.57%, 95% CI: 24.45%–65.50%), and erythromycin (39.85%, 95% CI: 19.58%–61.77%). Meta-regression identified the “all ages” participant category as a significant predictor of lower NT-Sp prevalence ($p = 0.034$). Molecular tools revealed substantial genomic diversity, with dominant clonal complexes varying by region.

Conclusion: NT-Sp is a globally distributed and genetically diverse pneumococcal subset with considerable AMR potential. Its persistence post-PCV introduction and resistance gene carriage highlights the need for improved detection, molecular surveillance, and

Abbreviations: AMR, antimicrobial resistance; CAP, community-acquired pneumonia; CI, confidence interval; DCC, day-care center; HIV, human immunodeficiency virus; IPD, invasive pneumococcal disease; MDR, multidrug resistance; MLST, multilocus sequence typing; NT-Sp, non-typeable *Streptococcus pneumoniae*; PCR, polymerase chain reaction; PCV, pneumococcal conjugate vaccine; PFGE, pulsed-field gel electrophoresis; PRISMA, Preferred Reporting Items for Systematic Reviews and Meta-Analyses; qPCR, quantitative polymerase chain reaction; ST, sequence type; WGS, whole-genome sequencing.

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consideration in next-generation vaccine development. Specifically, clinical laboratories in high-prevalence settings should adopt molecular methods (e.g., PCR or WGS) to detect NT-Sp in carriage surveillance; empirical antibiotic guidelines for pediatric respiratory infections should consider NT-Sp resistance patterns, particularly the high prevalence of co-trimoxazole resistance (67%) and next-generation PCV trials should include NT-Sp carriage as an exploratory endpoint. Integrating NT-Sp into global pneumococcal control strategies is essential.

1 | Introduction

Streptococcus pneumoniae is a Gram-positive pathobiont that colonizes the nasopharynx asymptomatically [1] yet remains a leading cause of invasive pneumococcal diseases (IPD), including pneumonia, meningitis, and bacteremia, particularly in children, the elderly, and immunocompromised individuals [2]. Globally, pneumococcal infections account for over 1.6 million deaths annually, with the highest burden in low-resource settings where vaccine access is limited [3, 4]. Key risk factors for severe IPD include young age, older age (≥ 65 years), asplenia, sickle cell disease, HIV infection, chronic heart or lung disease, and limited access to healthcare in low-resource settings [4, 5]. While over 100 serotypes have been identified based on capsular polysaccharides [5], non-typeable pneumococci (NT-Sp), strains lacking detectable capsules, represent a unique subset that challenges conventional serotyping methods and plays a complex role in carriage dynamics and disease [6, 7]. Although NT-Sp rarely causes invasive pneumococcal disease, several studies have documented its involvement in conjunctivitis outbreaks and occasional invasive disease cases [6, 7].

NT-Sp are frequently overlooked in surveillance due to their inability to elicit a Quellung reaction or agglutinate with standard antisera [6]. However, molecular techniques such as S2-gene sequencing and multilocus sequence typing (MLST) have confirmed their genetic identity as *S. pneumoniae*, revealing a prevalence of up to 55% in infant carriage studies [8]. Unlike encapsulated strains, NT-Sp rarely cause IPD but are increasingly recognized as reservoirs for antimicrobial resistance (AMR) genes and contributors to serotype replacement post-vaccination [9]. Their high recombination rates and genomic plasticity may facilitate the transfer of virulence factors or resistance determinants to encapsulated strains, complicating efforts to control pneumococcal disease [7, 9]. For example, Tavares et al. (2014) demonstrated that Portuguese NT-Sp strains (NCC2 clonal complex) carried the same pneumococcal surface protein A (PspA) family and pilus islets as encapsulated clones, suggesting frequent horizontal gene transfer. Similarly, Dayie et al. (2019) identified NT-Sp isolates in Ghana harboring *mefA* and *ermB* macrolide resistance genes also found in vaccine serotypes.

The rise of pneumococcal conjugate vaccines (PCVs) has dramatically reduced carriage of vaccine-serotype pneumococci [10], creating an ecological niche for non-vaccine strains. Within this niche, NT-Sp has emerged as a persistent colonizer, with several studies documenting stable or increased NT-Sp carriage post-PCV introduction [8, 11, 12]. This persistence may be explained by unique genetic features that enhance NT-Sp adaptation to the nasopharynx, including frequent absence of

the *piaB* gene (a marker for iron acquisition) and presence of *zmpC* (associated with mucosal colonization) [7]. These features, combined with high recombination rates, position NT-Sp as a potential reservoir for AMR genes and a contributor to serotype replacement.

Despite their clinical and epidemiological significance, NT-Sp remain understudied compared to encapsulated serotypes. Existing data on their sequence types (ST's), geographic distribution, and AMR profiles are fragmented, hindering a unified understanding of their public health impact. This systematic review synthesizes global evidence on NT-Sp carriage. By integrating molecular and clinical data, this review aims to inform vaccine strategies, antimicrobial stewardship, and surveillance protocols for NT-Sp in the PCV era.

2 | Methods

This systematic review followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) standards [13]. This review was not registered in PROSPERO; however, the protocol followed PRISMA guidelines throughout. All statistical analyses were performed using R (version 4.3.1) with the meta and metafor packages. The analysis plan followed the SAMPL (Statistical Analyses and Methods in the Published Literature) guidelines for reporting of statistical methods. Reporting of this systematic review followed the CONSORT (Consolidated Standards of Reporting Trials) guidelines where applicable. Statistical significance was set at $p < 0.05$, and all tests were two-sided. p -values were reported as follows: $p < 0.001$ as " $p < 0.001$ "; p values between 0.001 and 0.01 to the nearest thousandth; p -values ≥ 0.01 to the nearest hundredth; and p -values > 0.99 as " $p > 0.99$ ". The meta-analysis methodology adhered to the recommendations of Assel et al. (2019) [14] for reporting of statistics in clinical research. As this is a systematic review and meta-analysis of published literature, ethical approval and informed consent were not required.

2.1 | Literature Search Strategy and Selection Process

Using keywords associated with *Streptococcus pneumoniae* non-typeable, a comprehensive literature search was carried out in Google Scholar, PubMed, Scopus, Web of Science, and ScienceDirect. Only free full-text or open-access research articles published in English were included in the search, which ran until May 2025 (Table 1). Records that were retrieved were exported in CSV and RIS formats.

TABLE 1 | Detailed search keywords and filters used in each database.

Database	Keywords	Filters		
		Language	Document type	Open access
PubMed	("Streptococcus pneumoniae" OR pneumococcus) AND ("non-typeable" OR "non-typeable strains" OR "untypable" OR "non-serotypeable" OR "NT")	English	—	Free full-text
Scopus		English	Article	All open access
ScienceDirect		English	Research article	Open access & open archives
Web of Science				
Google Scholar		English	—	—

The Rayyan application [15], a web-based tool for systematic reviews, was utilized to screen the citations of the acquired records. The remaining unique records were filtered by title and abstract after duplicates were found and removed, and any records that were deemed unnecessary were removed as well. The remaining articles were assessed for full-text eligibility using the aforementioned inclusion criteria after the titles and abstracts were screened. The included studies were imported into Zotero 7.0.8 in order to extract the data.

2.2 | Eligibility Criteria

Eligible studies were observational (cross-sectional, cohort, or surveillance) or clinical trials that reported carriage or antimicrobial resistance (AMR) of non-typeable *Streptococcus pneumoniae* (NT-Sp). Participants included healthy or infected individuals of any age. Included studies had to identify *S. pneumoniae* using phenotypic methods (e.g., Quellung reaction and optochin sensitivity) or molecular approaches such as PCR, MLST, or whole-genome sequencing (WGS), and provide explicit NT-Sp data, including resistance rates and multidrug resistance profiles.

For this review, NT-Sp was operationally defined as pneumococcal isolates lacking detectable capsular serotype by phenotypic serotyping methods (e.g., Quellung reaction or latex agglutination) and/or isolates confirmed as non-encapsulated through molecular approaches such as cpsA-negative PCR, lytA-positive PCR, MLST, or WGS. Variability in diagnostic criteria across studies was considered a potential source of heterogeneity.

Excluded studies were case reports, reviews, editorials, comments, non-peer-reviewed sources (e.g., theses, dissertations, and preprints), and studies that focused on other serotypes or did not provide specific NT-Sp carriage data.

2.3 | Risk of Bias Assessment

Two researchers (R.K.R. and E.S.D.) independently assessed the methodological quality of included studies using the Newcastle–Ottawa Scale [16] (NOS) adapted for cross-sectional studies. The NOS evaluates studies across three domains: selection (representativeness, sample size, non-respondents, and exposure ascertainment), comparability (control for confounding), and outcome (outcome assessment and statistical

analysis). A maximum of 8 stars could be awarded, with studies scoring 7–8 stars classified as high quality, 5–6 stars as moderate quality, and ≤ 4 stars as low quality. Disagreements between reviewers were resolved through consensus. Of the 40 included studies, 34 (85%) were rated as high quality (7–8 stars), and six (15%) were rated as moderate quality (5–6 stars). No studies were rated as low quality. The most common methodological limitations were lack of non-respondent description and limited control for confounding factors. The full risk of bias assessment is presented in Table 2.

2.4 | Data Extraction and Analysis

The relevant data were extracted from each included study as described in Table 3 by R.K.R. and E.S.D. *S. pneumoniae* non-typeable carriage data were extracted and stratified by country. Microsoft Excel 2016 was used to extract and manage the extracted data.

2.5 | Statistical Analysis

Meta-analysis was conducted using a random-effects model to estimate pooled prevalence and resistance proportions because substantial clinical and methodological heterogeneity was anticipated across studies, including differences in study populations, geographic settings, vaccination eras, and laboratory identification methods. Heterogeneity was assessed using the I^2 statistic. Publication bias was evaluated using funnel plots and Egger's regression test. Subgroup analyses (by age, region, and vaccination era) and meta-regression were performed to explore potential sources of heterogeneity. All analyses were conducted in R using the meta and metafor packages.

3 | Results

3.1 | Search Results and Selection Process

A total of 985 records were identified from all four electronic databases. Two hundred ninety duplicate records were removed, leaving 695 unique records to be screened. Of the 695 unique records, 566 were excluded based on their title, abstract, and language, and the remaining 129 records were sought for full-text retrieval. After full-text retrieval ($n = 129$), 67 articles were excluded following the full-text eligibility assessment. Finally, 40 studies were included in the systematic review (Figure 1).

TABLE 2 | Risk of bias assessment for included studies.

Study (year)	Representativeness	Sample size	Non-respondents	Exposure ascertainment	Comparability	Outcome assessment	Statistical analysis	Total stars	Quality
Turner et al., 2012	★	★	★	★	★★	★	★	8	High
Usuf et al., 2021	★	★	★	★	★★	★	★	8	High
Wyllie et al., 2017	★	★	★	★	★★	★	★	8	High
Sá-Leão et al., 2009	★	★	★	★	★	★	★	7	High
Rybák et al., 2022	★	★	—	★	★★	★	★	7	High
Pinto et al., 2019	★	★	★	★	★	★	★	7	High
Andrade et al., 2010	★	★	★	★	★★	★	★	8	High
Ousmane et al., 2017	★	★	★	—	★	★	★	6	Moderate
Mills et al., 2020	★	★	★	★	★	★	★	7	High
Dayie et al., 2018	★	★	★	★	★★	★	★	8	High
Dayie et al., 2019	★	★	★	★	★	★	★	7	High
McElligott et al., 2014	★	★	★	★	★	★	★	7	High
Lindstrand et al., 2016	★	★	★	—	★	★	★	6	Moderate
Lansbury et al., 2024	★	★	★	★	★★	★	★	8	High
Kamng'ona et al., 2015	★	★	★	★	★★	★	★	8	High
Adetifa et al., 2018	★	★	★	★	★	★	★	7	High
Jourdain et al., 2011	★	★	★	★	★	★	★	7	High
Ziane et al., 2016	★	★	★	★	★	★	★	7	High
Wróbel-Pawelczyk et al., 2022	★	★	★	★	★	★	★	7	High
Vestrhein et al., 2008	★	★	★	★	★	★	★	7	High
Sharma et al., 2013	★	★	★	★	★	★	★	7	High
Satzke et al., 2018	★	★	★	★	★★	★	★	8	High
Safari et al., 2014	★	★	—	★	★	★	★	6	Moderate
Orami et al., 2023	★	★	★	★	★	★	★	7	High
Njuma Libwea et al., 2020	★	★	★	★	★	★	★	7	High
Neves et al., 2013	★	★	★	★	★	★	★	7	High

(Continues)

TABLE 2 | (Continued)

Study (year)	Representativeness	Sample size	Non-respondents	Exposure ascertainment	Comparability	Outcome assessment	Statistical analysis	Total stars	Quality
Narworte y et al., 2021	★	★	★	★	★	★	★	7	High
Meats et al., 2003	★	★	★	★	★	★	★	7	High
Ercibengoa et al., 2012	★	★	★	★	★	★	★	7	High
Dunne et al., 2016	★	★	★	★	★★	★	★	8	High
Dhoubhadel et al., 2014	★	★	★	★	★	★	★	7	High
da Silva et al., 2023	★	★	★	★	★★	★	★	8	High
Chamorro et al., 2023	★	★	★	★	★	★	★	7	High
Britton et al., 2021	★	★	★	★	★	★	★	7	High
Ben Ayed et al., 2024	★	★	★	★	★	★	★	7	High
Azzari et al., 2016	★	★	★	★	★	★	★	7	High
Auranen et al., 2010	★	★	★	★	★	★	★	7	High
Anthony et al., 2012	★	★	★	—	★	★	★	6	Moderate
Adetifa et al., 2012	★	★	★	★	★	★	★	7	High
Hotomi et al., 2016	★	★	★	★	★	★	★	7	High

TABLE 3 | Characteristics of included studies.

Author, year	Country	Continent	Year of study	Study population	Study group	No. of <i>S. Pneumoniae</i>			Molecular identification method/ genotyping tools	Vaccination era
						non-typeable carriage	Total samples tested			
Usuf et al., 2021 [11]	The Gambia	Africa	2003–2016	Individuals from western region of the Gambia	All ages	89	121	WGS, MLST		Post-PCV13 and PCV7
McElligott et al., 2014 [17]	Ireland	Europe	2007–2012	Carriage (nasopharyngeal/nasal/throat swabs), NII (conjunctivitis, nLRTI, and OM)	Children	13	339	PCR, Quellung reaction		PCV7 (2008), PCV13 (2010) introduction
Wyllie et al., 2017 [18]	Netherlands	Europe	2005–2015	Asymptomatic individuals	Children (≤ 2 years), older adults (≥ 60 years)	17	221	S2-gene sequencing (rpsB), MLST		Post-PCV7/PCV13 introduction
Turner et al., 2012 [8]	Thailand–Myanmar Border	Asia	2007–2008	Karen mother–infant pairs	Infants (0–24 months) and mothers	660	8386	Latex agglutination, Quellung reaction, PCR, bile solubility, MLST		Pre-PCV13 introduction
Sa-Leao et al., 2009 [12]	Portugal	Europe	2006	Day-care center attendees from Lisbon and Oeiras	Children (4 months to 6 years)	27	1288	Multiplex PCR, Quellung reaction, MLST		Mixed (pre- and post-PCV7 introduction)
Rybak et al., 2022 [19]	France	Europe	2017	Children Attending the Same Day-Care Center	Children (3 years and younger)	3	29	Latex Agglutination, (MLST)		Post-PCV13 implementation (since 2010 in France)
Pinto et al., 2019 [20]	Brazil	South America	1990–2014	Asymptomatic carriers and IPD patients	Children and adults	13	783	Multiplex PCR, Quellung reaction		Pre- and post-PCV10 introduction (PCV10 introduced in Brazil in 2010)
Ousmane et al., 2017 [21]	Niger	Africa	2007–2008	Asymptomatic nasopharyngeal carriers	Children (0–2 years)	62	654	Multiplex PCR		Pre-PCV13 introduction (PCV13 introduced in Niger in July 2014)
Mills et al., 2020 [22]	Ghana	Africa	2018	Children from kindergartens and immunization	Children < 5 years	1	513	Multiplex PCR, Quellung reaction		Post-PCV13 introduction

(Continues)

TABLE 3 | (Continued)

Author, year	Country	Continent	Year of study	Study population	Study group	No. of <i>S. Pneumoniae</i> non-typeable carriage	Total samples tested	Molecular identification method/ genotyping tools	Vaccination era
Lindstrand et al., 2016 [23]	Uganda	Africa	2008, 2009, and 2011	centers in Cape Coast Rural and peri-urban communities in Iganga/Mayuge Health and Demographic Surveillance Site	Children < 5 years	9	927	Gel diffusion and/or Quellung reaction	Pre-PCV introduction (PCV introduced in Uganda in 2014)
Lansbury et al., 2024 [24]	UK	Europe	2016–2018	Hospitalized adults with community-acquired pneumonia (CAP)	Adults aged ≥ 16 years old	11	258	PCR	Post-PCV13 introduction
Kamng'ona et al., 2015 [25]	Malawi	Africa	2008–2012	Rural (Karonga) and urban (Blantyre) communities; outpatient and household recruitment	Children aged 0–13 years	2	189	WGS	Pre-PCV13 introduction
Dayie et al., 2019 [26]	Ghana	Africa	2016	Nursery/Kindergarten	Children ≤ 5 years	10	410	WGS (Illumina), MLST, PBP	Post-PCV-13 (2012)
Dayie et al., 2018 [27]	Ghana	Africa	2016–2017	SCD patients (children & adults)	Children (< 13 yrs) & Adults (> 13 yrs)	5	402	Latex agglutination, Quellung reaction	Post-PCV-13 (2012)
Andrade et al., 2010 [28]	Brazil	South America	2005–2008	Children in day-care centers (DCCs) & IPD surveillance	Children (2–59 months) & IPD cases (< 3 years)	22	133	PCR, Quellung reaction, PFGE, MLST	Pre-PCV-10 (introduced 2010)
Adetifa et al., 2018 [29]	Nigeria	Africa	2016–2017	Rural and urban populations	Children and Adults	47	1800	Multiplex PCR, Quellung reaction, Latex agglutination	Post-PCV10 introduction
Jourdain et al., 2011 [30]	Belgium	Europe	2006–2008	Healthy preschool children	Children (3–6 years)	28	830	Quellung reaction, bile solubility, <i>lytA</i> PCR confirmation for NTP	Early PCV7 (introduced 2004; free from 2007)

(Continues)

TABLE 3 | (Continued)

Author, year	Country	Continent	Year of study	Study population	Study group	No. of <i>S. Pneumoniae</i> non-typeable carriage	Total samples tested	Molecular identification method/ genotyping tools	Vaccination era
Ziane et al., 2016 [31]	Algeria	Africa	2010–2014 (IPD); 2011–2012 (carriage)	IPD cases and asymptomatic carriers	Children < 5 years (healthy + IPD)	20	218	Sequential multiplex PCR, Quellung reaction, <i>lytA</i> PCR for NTP	Pre-vaccination era
Wróbel-Pawelczyk et al., 2022 [32]	Poland	Europe	2016–2020	Outpatients (DCC/ home visits)	Children aged 12–59 months, unvaccinated	6	394	qPCR targeting <i>piaB</i> and <i>lytA</i> genes; Culture + serotyping (ImmuLex, PCR/ sequencing), Quellung	Early implementation of PCV10 (NIP started 2017)
Vestheim et al., 2008 [33]	Norway	Europe	2006	Children	Children (10–69 months) attending 29 day-care centers	24	573	Quellung reaction, latex agglutination (Pneumotest-Latex), MLST, optochin sensitivity, E-test for MICs	Pre-vaccination (before PCV-7 introduction)
Sharma et al., 2013 [34]	USA	North America	1995 and 2009	Outpatients (carriage); IPD cases (lab-based)	Children aged 6–59 months	3	682	Quellung reaction, MLST, broth enrichment, PCR for serotype 6 C, broth microdilution MICs (CLSI 2008 criteria)	1995: Pre-PCV7 2009: Pre-PCV13
Satzke et al., 2018 [35]	Lao PDR	Asia	2013–2016	Pre- and post-PCV13 cross-sectional surveys	Children aged 5–8 weeks and 12–23 months	112	2010	<i>lytA</i> qPCR, culture + Senti-SPv1.5 DNA microarray, latex agglutination	Early PCV13 era (introduced 2013)
Safari et al., 2014 [36]	Indonesia	Asia	2012	Outpatient HIV clinic attendees	HIV-infected children (aged 4–144 months)	11	90	<i>psaA</i> PCR, Sequential multiplex PCR (smPCR), <i>cpsA</i> gene PCR	Pre-PCV13
Orami et al., 2023 [37]	Papua New Guinea	Oceania	2011–2016	Randomized PCV10 vs. PCV13 + PPV/no PPV at 9 months	Children aged 1–23 months	144	1063	Quellung reaction, Kirby–Bauer disc diffusion, MIC (E-test), standard culture	Early PCV13 rollout

(Continues)

TABLE 3 | (Continued)

Author, year	Country	Continent	Year of study	Study population	Study group	No. of <i>S. Pneumoniae</i> non-typeable carriage	Total samples tested	Molecular identification method/ genotyping tools	Vaccination era
Njuma Libwea et al., 2020 [38]	Cameroon	Africa	2013 and 2015	Children	Children aged 24–36 months	60	887	Culture, optochin sensitivity, sequential multiplex PCR (mPCR), Quellung test, <i>lytA</i> PCR for <i>cpsA</i> -negative isolates	Post-PCV13
Neves et al., 2013 [39]	Brazil	South America	2010	DCC and Pediatric Hospital (PH) attendees	Children < 6 years	4	242	Sequential multiplex PCR, Quellung reaction, <i>lytA</i> PCR (for <i>cpsA</i> -negative/NT confirmation)	Pre-PCV10
Narworthey et al., 2021 [40]	Ghana	Africa	2018	Random community sample	Healthy children aged 5–12 years	9	193	Latex agglutination, Quellung reaction, Biochemical ID (optochin sensitivity, bile solubility),	Post-PCV13 (introduced 2012)
Meats et al., 2003 [41]	United Kingdom	Europe	1999–2001	Longitudinal monthly/bi-monthly sampling	Birth cohort of 100 children	4	1353	MLST, Quellung reaction, optochin sensitivity, bile solubility, pneumolysin PCR	Pre-PCV7 (introduced in 2006 in the UK)
Ercibengoa et al., 2012 [42]	Spain	Europe	2004–2005	Daycare attendees in northern Spain	Healthy children aged 6–34 months	13	208	Quellung reaction, Pneumotest-Latex, multiplex PCR, <i>lytA</i> gene PCR, PFGE, and MLST	Early PCV7 (introduced 2001)
Dunne et al., 2016 [43]	Australia	Oceania	1999–2005	Kalgoorlie Otitis Media Research Project birth cohort	Children aged 0–2 years	23	1551	Quellung reaction, disk diffusion, Etest, PFGE, MLST	Early PCV7
Dhoubhadel et al., 2014 [44]	Vietnam	Asia	2008–2009	ARI cases and healthy children	Children < 5 years	33	945	<i>lytA</i> PCR, nanofluidic real-time PCR system	Post-PCV7
da Silva et al., 2023 [45]	Brazil	South America	2016	Oropharyngeal carriers and non-carriers	Adults ≥ 18 years	11	385	Multiplex PCR for serotyping, <i>lytA</i> PCR for pneumococcal identification	Post-PCV10 (introduced in 2010)

(Continues)

TABLE 3 | (Continued)

Author, year	Country	Continent	Year of study	Study population	Study group	No. of <i>S. Pneumoniae</i> non-typeable carriage	Total samples tested	Molecular identification method/ genotyping tools	Vaccination era
Chamorro et al., 2023 [46]	Paraguay	South America	2012	Healthy outpatients	Children 2–59 months, Adults ≥ 60 years	Not explicitly reported	1444	Quellung reaction, Multiplex PCR, enrichment culture, optochin and bile solubility tests	Pre-PCV10 introduction
Britton et al., 2021 [47]	Papua New Guinea	Oceania	2011–2016	Vaccinated (PCV13) & Unvaccinated infants	Infants aged 0–11 months	33	333	qPCR (<i>lytA</i>), DNA microarray (Senti-SP v1.5), enrichment culture, serotype-specific PCR	Post-PCV13 introduction (2014)
Ben Ayed et al., 2024 [48]	Tunisia	Africa	2020–2022	Attendees of kindergartens & clinics	Healthy children under 5 years	9	1256	<i>lytA</i> PCR, <i>cpsA</i> PCR, sequential multiplex & simplex PCR, latex agglutination	Early PCV10 era (introduced 2019)
Azzari et al., 2016 [49]	Italy	Europe	2007–2014	Adults with IPD and healthy children	Adults with IPD; healthy children (0–60 months)	18	629	RT-PCR (<i>lytA</i> , <i>cpsA</i> genes), multiplex serotyping	PCV7 then PCV13 (since July 2010)
Auranen et al., 2010 [50]	Denmark	Europe	1999–2001	Unvaccinated children in 3 nurseries	Children in day-care centers (ages 0.9–3.2 years)	14	571	Capsular reaction test with group/type-specific antisera	Pre-PCV (introduced 2007)
Anthony et al., 2012 [51]	Tanzania	Africa	2008	HIV-infected, unvaccinated	HIV-positive children (1–14 years)	13	142	Culture, Quellung reaction, PCR (<i>lytA</i>), mPCR/RLB, <i>cpsA</i> –B sequencing	Pre-PCV
Adetifa et al., 2012 [52]	Nigeria	Africa	Not specified (cross-sectional)	Unvaccinated, pre-PCV era	Peri-urban community (all ages)	17	1005	Culture, latex agglutination, multiplex PCR, MLST	Pre-PCV introduction
Hotomi et al., 2016 [53]	Japan	Asia	2006–2013	Children with AOM, rhinosinusitis, and healthy carriers	Ages 2–158 months	20	275	PCR, MLST, Quellung reaction	Post-PCV7 introduction (limited vaccination)

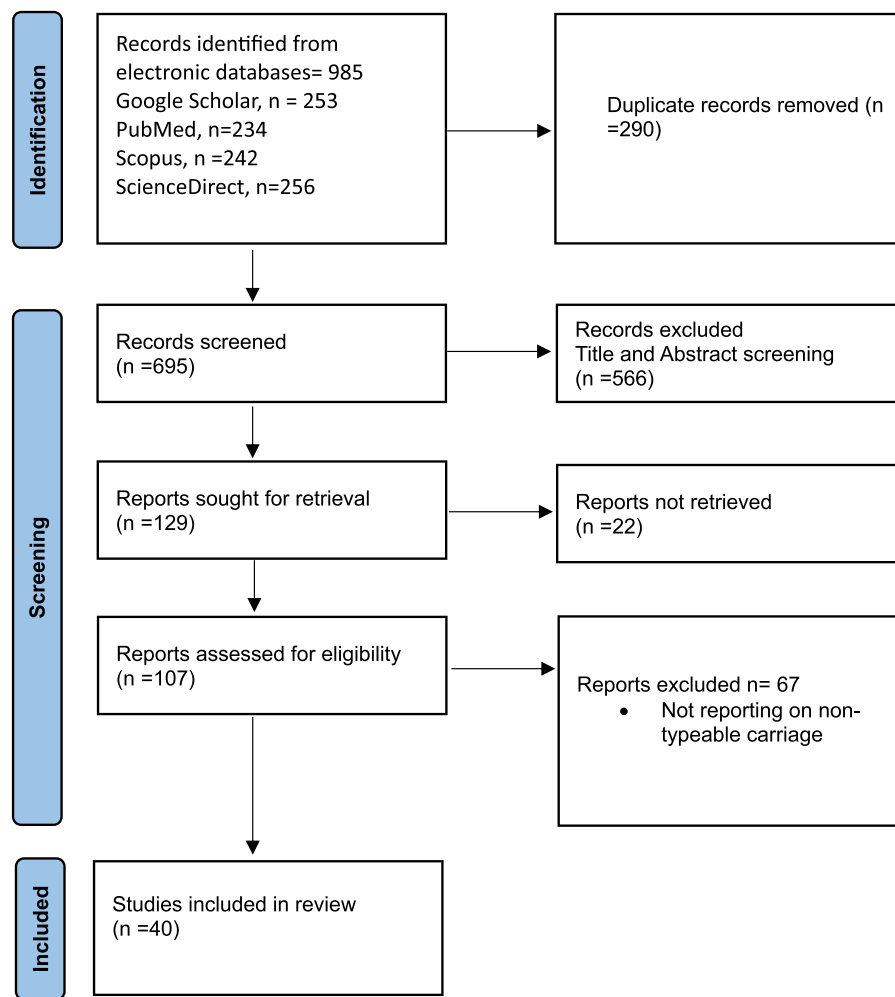


FIGURE 1 | PRISMA flow diagram of the study selection process.

3.2 | Description of Included Studies

This systematic review included 40 studies from 23 countries across six continents (Table 3). The geographic distribution revealed a dominance of African ($n=13$) and European ($n=12$) studies, followed by South America ($n=5$), Asia ($n=6$), Oceania ($n=3$), and North America ($n=1$). Ghana contributed the most studies ($n=4$), followed by Brazil ($n=3$) and Portugal ($n=2$). Most investigations were single-center studies focusing on community or hospital-based sampling.

The study populations were diverse: 10 studies sampled only children, six included both children and adults, one focused exclusively on adults (≥ 16 years), and two used mother–infant pairs. The remaining studies included mixed populations or did not explicitly stratify by age. Over 70% of the included studies were conducted post-PCV introduction, spanning PCV7, PCV10, and PCV13 eras. Sample sizes ranged from under 100 to over 8000 individuals.

Non-typeable *Streptococcus pneumoniae* (NT-Sp) carriage prevalence ranged from 0.19% to 73.55%, with higher rates generally observed among infants and in pre-vaccine populations.

3.2.1 | Prevalence of Non-Typeable Pneumococcal Carriage in the Isolates

The pooled prevalence of non-typeable *Streptococcus pneumoniae* (NT-Sp) carriage was estimated using a random-effects meta-analysis. Figure 2 presents the overall pooled prevalence together with the individual study estimates and measures of between-study heterogeneity.

3.2.2 | Subgroup Analysis by Geographic Region

To investigate whether geographic location contributed to the observed heterogeneity, subgroup analyses were performed according to continent. The pooled prevalence estimates for each geographic region are presented in Figure 3.

3.2.3 | Age Group of Study Participants

Subgroup analysis was also conducted according to the age category of study participants to assess potential differences in NT-Sp carriage across populations. The pooled prevalence estimates for each age group are shown in Figure 4.

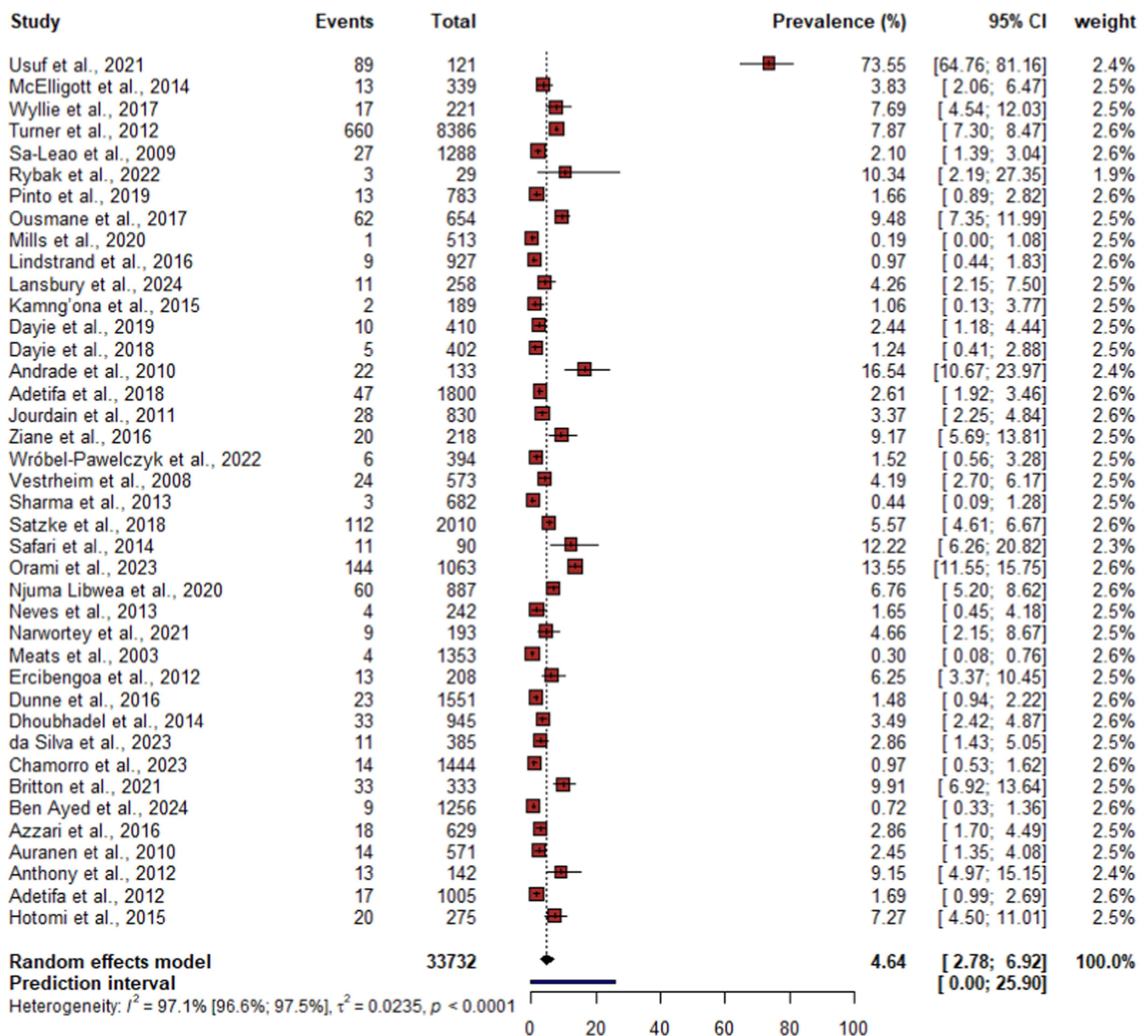


FIGURE 2 | Forest plot showing the pooled prevalence of NT-Sp carriage from 40 included studies using a random-effects model. Pooled prevalence: 4.64% (95% CI: 2.78%–6.92%). Heterogeneity: $I^2 = 97.1\%$ (95% CI: 96.6%–97.5%), $\tau^2 = 0.0035$, $p < 0.0001$. Prediction interval: 0.00%–25.90%. Each horizontal line represents the confidence interval (CI) for an individual study, while the diamond indicates the overall summary estimate.

3.2.4 | Vaccination Period

To evaluate the influence of pneumococcal conjugate vaccine (PCV) implementation on NT-Sp carriage, studies were stratified according to vaccination period (pre-vaccination, post-vaccination, and mixed periods). The corresponding pooled prevalence estimates are presented in Figure 5.

3.2.5 | Publication Bias

Potential publication bias among the included studies was assessed using a funnel plot and Egger's regression test. The results of this assessment are presented in Figure 6.

3.2.6 | Detection Method Subgroup Analysis

Because laboratory identification methods varied across studies, subgroup analyses were performed according to the primary detection method used for NT-Sp identification. The pooled prevalence estimates for each detection method are shown in Figure 7.

3.2.7 | Sensitivity Analysis

A leave-one-out sensitivity analysis was conducted to assess the robustness of the pooled prevalence estimate and determine whether any individual study had a disproportionate influence on the overall results. The findings are summarized in Figure 8.

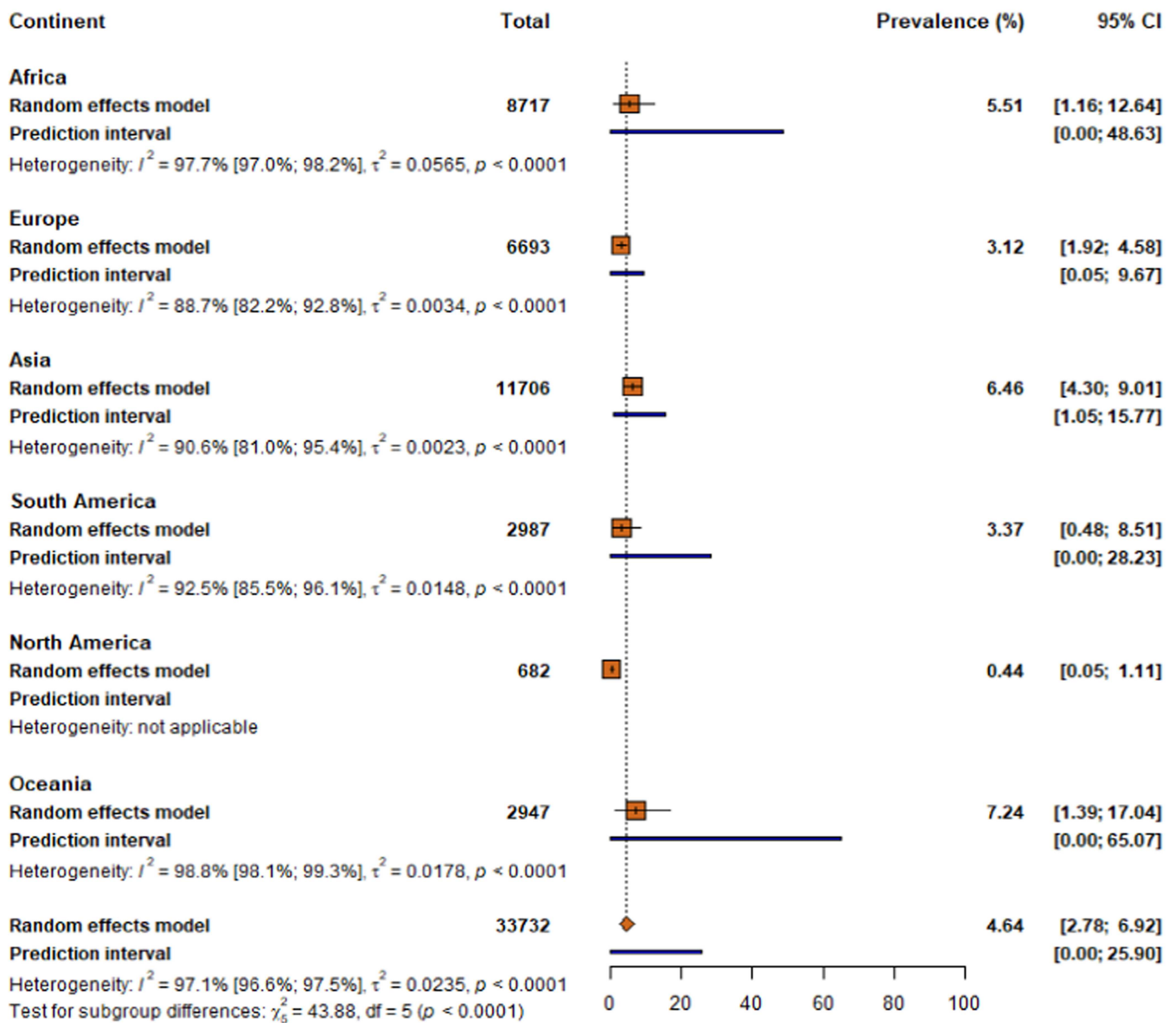


FIGURE 3 | Forest plot showing NT-Sp carriage prevalence stratified by geographic region. Asia: 6.46% (95% CI: 4.30%–9.01%, $I^2 = 90.6\%$); Oceania: 7.24% (95% CI: 1.39%–17.04%, $I^2 = 98.8\%$); Africa: 5.51% (95% CI: 1.16%–12.64%, $I^2 = 97.7\%$); South America: 3.37% (95% CI: 0.48%–8.51%, $I^2 = 92.5\%$); Europe: 3.12% (95% CI: 1.92%–4.58%, $I^2 = 88.7\%$); North America: 0.44% (95% CI: 0.05%–1.11%). Test for subgroup differences: $\chi^2 = 43.88$, $df = 5$, $p < 0.001$. Overall pooled prevalence: 4.64% (95% CI: 2.78%–6.92%).

3.2.8 | Meta-Regression

Meta-regression analyses were performed using univariable random-effects models to explore potential sources of heterogeneity in NT-Sp carriage prevalence. Covariates included age category, vaccination period, geographic region, detection method, and sample size. Categorical variables were dummy-coded prior to analysis. Due to the limited number of studies within some subgroups, multivariable modeling was not performed to avoid model overfitting and unstable estimates.

3.2.9 | Antibiotic Resistance in the Isolates

The pooled prevalence of antimicrobial resistance among NT-Sp isolates was estimated for the most commonly reported antibiotics using random-effects meta-analysis. The resistance estimates for each antimicrobial agent are presented in Figure 9.

3.2.10 | Molecular Tools and Genetic Diversity

Molecular characterization of NT-Sp isolates revealed substantial diversity across geographic regions. Whole-genome sequencing (WGS), multilocus sequence typing (MLST), and PCR-based assays were the predominant molecular tools used. These methods enabled confirmation of pneumococcal identity in *cpsA*-negative strains and differentiation from other commensal streptococci.

Clonal complexes such as ST66 were identified in Brazil, and ST320 in Ghana [26], illustrating regional specificity. In Southeast Asia and parts of Africa, studies noted the presence of non-encapsulated clones lacking traditional capsule biosynthesis genes but retaining key virulence and resistance determinants.

Genomic analyses demonstrated that NT-Sp strains shared up to 90.6% core genome similarity with encapsulated strains,

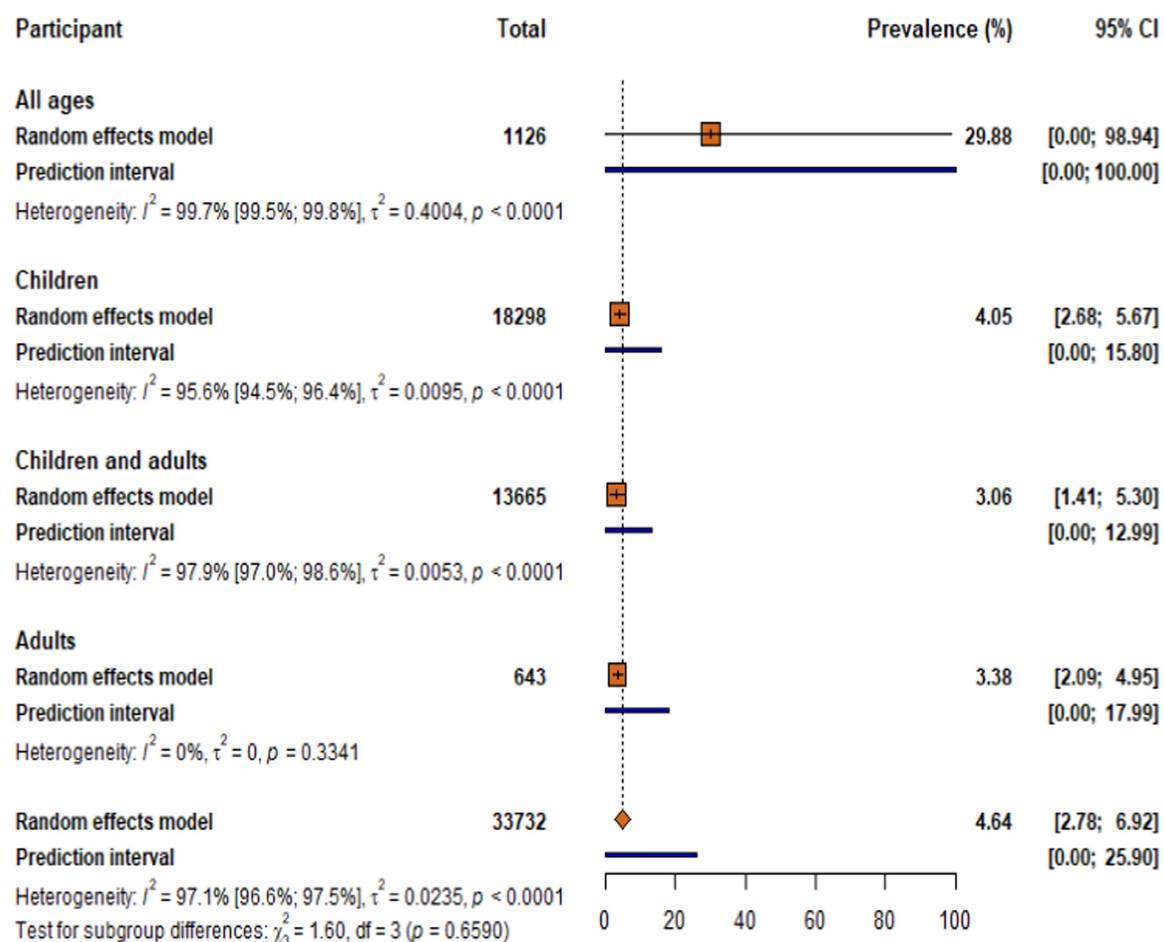


FIGURE 4 | Forest plot displaying NT-Sp carriage prevalence stratified by age group of participants. All ages: 29.88% (95% CI: 0.00%–100.00%, $I^2 = 99.7\%$); Children: 4.05% (95% CI: 0.00%–15.80%, $I^2 = 95.6\%$); Children and adults: 3.06% (95% CI: 0.00%–12.99%, $I^2 = 97.9\%$); Adults: 3.38% (95% CI: 0.00%–17.99%, $I^2 = 0\%$). Test for subgroup differences: $\chi^2 = 1.60$, $df = 3$, $p = 0.659$ (not significant). Overall pooled prevalence: 4.64% (95% CI: 0.00%–25.90%).

though lacking capsule synthesis loci. The absence of the *cps* locus (capsule biosynthesis genes) is the defining molecular feature of NT-Sp, consistent with observations that capsule loss can occur through recombination-mediated deletion events [7, 12]. This genetic adaptation may represent an evolutionary strategy to evade vaccine-induced immunity while retaining colonization fitness. Accessory gene content, including prophages, surface proteins, and AMR genes, varied significantly, reflecting adaptation to specific ecological niches. Some NT-Sp strains carried resistance determinants such as *mefA*, *ermB*, and *tetM*, further underscoring their role in antimicrobial resistance gene dissemination.

Standardization remains a challenge, as older serotyping methods frequently misclassified NT-Sp. The review highlights the need for consistent application of WGS and MLST, especially in low-resource settings, to ensure accurate surveillance.

4 | Discussion

This systematic synthesis highlights the global distribution and characteristics of non-typeable *Streptococcus pneumoniae* (NT-Sp), an often-overlooked component of pneumococcal ecology. Drawing from 40 studies across six continents, this review offers

the most comprehensive overview of NT-Sp carriage to date. While traditionally marginalized in surveillance due to their lack of polysaccharide capsules, NT-Sp strains emerge here as resilient colonizers with significant implications for antimicrobial resistance (AMR), vaccine strategy, and diagnostics [6, 7, 30, 33, 34]. The pooled global NT-Sp carriage prevalence of 4.64% (95% CI: 2.78%–6.92%) confirms that NT-Sp is a widespread colonizer, though the substantial heterogeneity ($I^2 = 97.1\%$, 95% CI: 96.6%–97.5%) underscores the influence of geographic, demographic, and methodological factors on observed prevalence estimates (Figure 2).

Carriage prevalence varied significantly by geographic region ($p < 0.001$). Oceania showed the highest pooled prevalence at 7.24% (95% CI: 1.39%–17.04%) (Figure 3), though with wide confidence intervals reflecting heterogeneity across studies from Papua New Guinea and Australia. Asia followed closely at 6.46% (95% CI: 4.30%–9.01%), with particularly high rates in Southeast Asia especially along the Thailand–Myanmar border where Turner et al. [8] reported NT-Sp carriage exceeding 50% among infants. This exceptionally high prevalence likely reflects multiple interacting factors: high transmission intensity due to crowding and large household sizes, limited PCV access during the study period (2007–2008), and the young infant cohort (0–24 months) that is most susceptible to pneumococcal

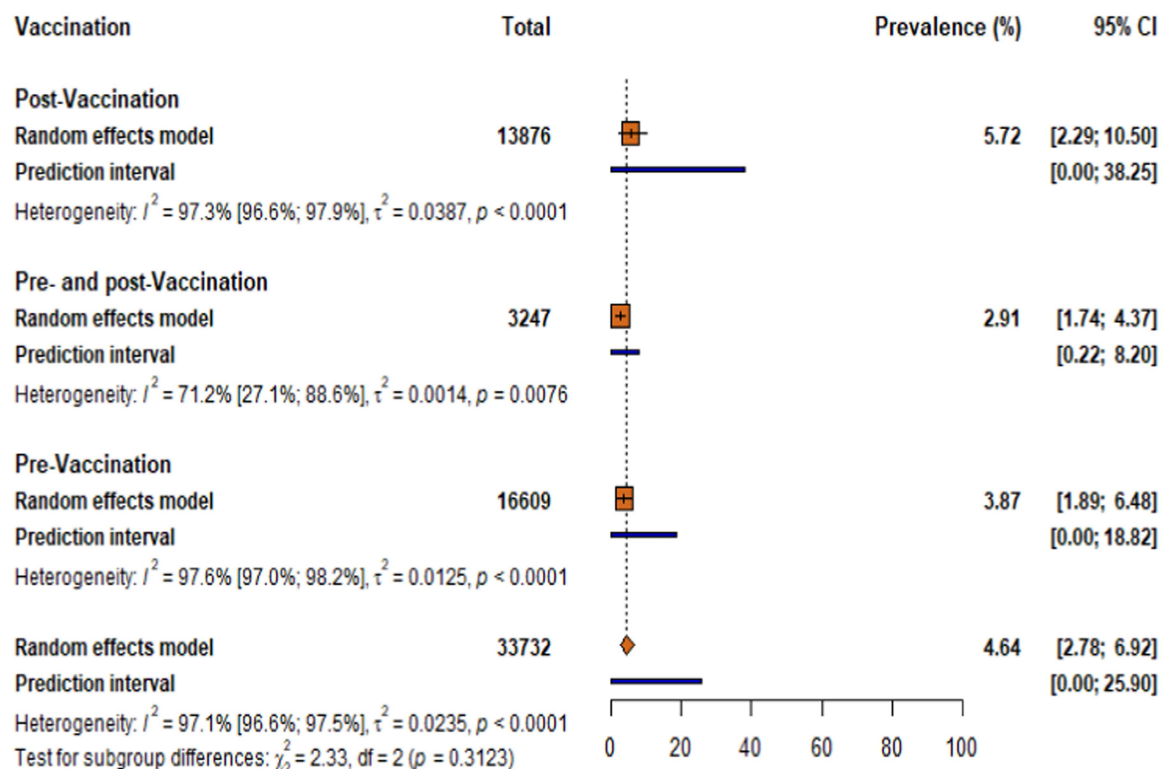


FIGURE 5 | Forest plot showing NT-Sp carriage prevalence stratified by vaccination era. Post-vaccination: 5.72% (95% CI: 2.29%–10.50%, $I^2 = 97.3\%$, $n = 13,876$); Pre- and post-vaccination (Mixed): 2.91% (95% CI: 1.74%–4.37%, $I^2 = 71.2\%$, $n = 3,247$); Pre-vaccination: 3.87% (95% CI: 1.89%–6.48%, $I^2 = 97.6\%$, $n = 16,609$). Test for subgroup differences: $\chi^2 = 2.33$, $df = 2$, $p = 0.312$ (not significant). Overall pooled prevalence: 4.64% (95% CI: 2.78%–6.92%).

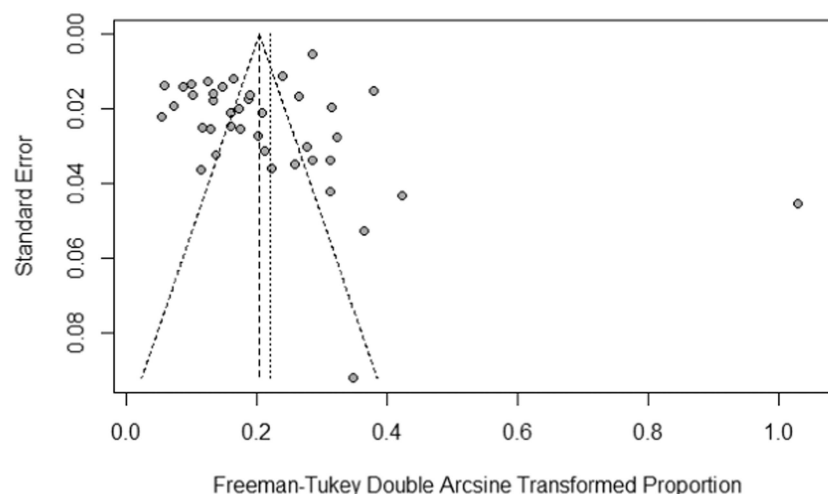


FIGURE 6 | Funnel plot assessing publication bias among included studies. The symmetrical distribution of points suggests an absence of significant publication bias. Egger's regression: Test result: $t = -0.23$, $df = 38$, $p\text{-value} = 0.8212$. Bias estimate: -0.4330 (SE = 1.9028).

acquisition. In contrast, European and North American studies reported the lowest NT-Sp carriage at 3.12% (95% CI: 1.92%–4.58%) and 0.44% (95% CI: 0.05%–1.11%), respectively, attributable to high PCV coverage ($\geq 85\%$ in most countries), better hygiene infrastructure, smaller household sizes, and potentially different circulating clones with lower colonization fitness. West African countries (The Gambia, Ghana, and Nigeria) showed intermediate prevalence ranging from 5% to 15% in individual studies, reflecting moderate PCV coverage but persistent high transmission intensity in urban and peri-urban

settings. Brazil showed varied prevalence (2%–10%) depending on pre- vs. post-PCV10 sampling periods [20, 28, 39, 45], suggesting that PCV introduction may have influenced NT-Sp ecology in complex ways.

The age-stratified analysis revealed that the “All ages” category had the highest pooled estimate at 29.88% (95% CI: 0.00%–100.00%) (Figure 4), though this finding must be interpreted with extreme caution due to the very wide confidence interval reflecting substantial heterogeneity ($I^2 = 99.7\%$) and

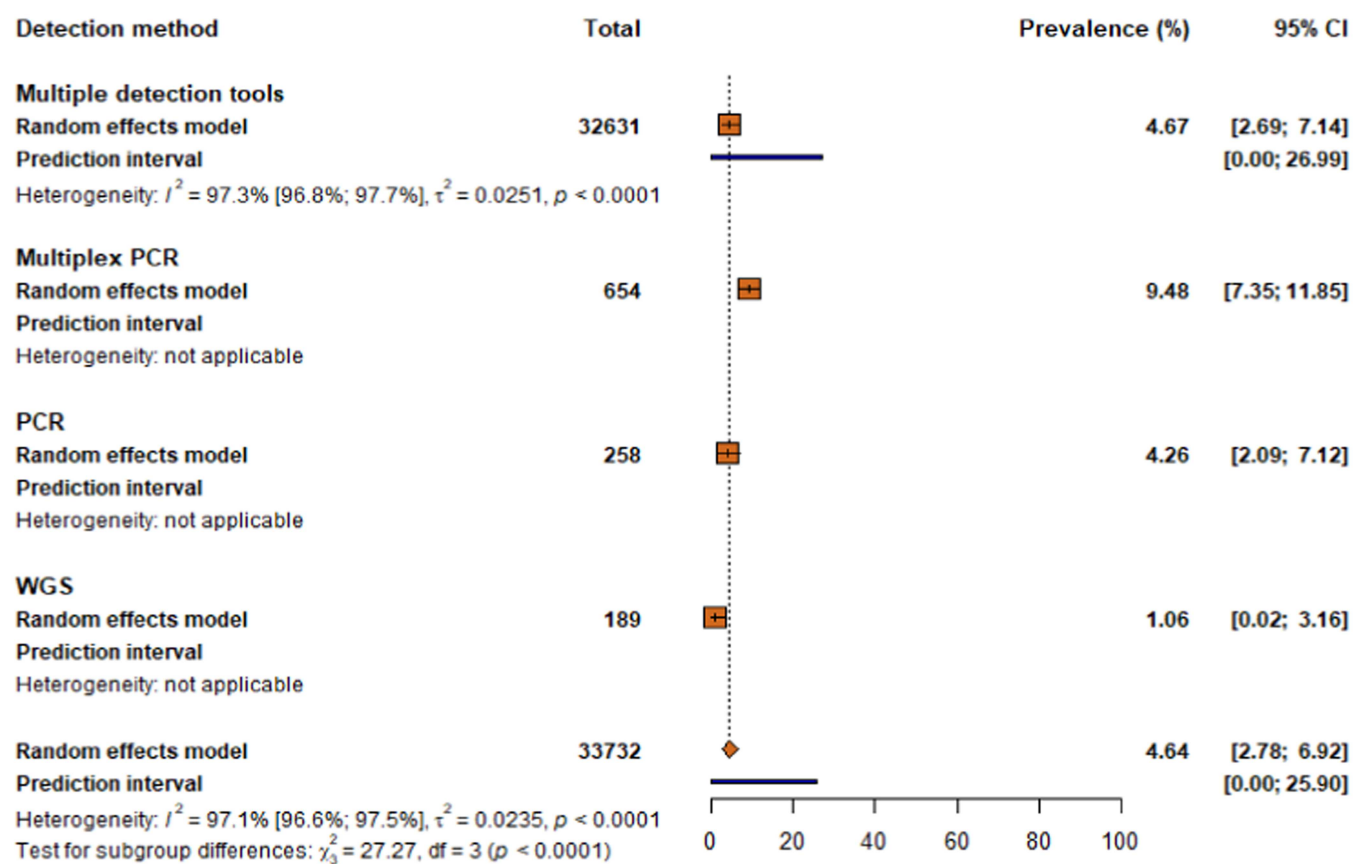


FIGURE 7 | Forest plot showing NT-Sp carriage prevalence stratified by detection method. Multiplex PCR: 9.48% (95% CI: 7.35%–11.85%); Multiple detection tools: 4.67% (95% CI: 2.69%–7.14%, $I^2 = 97.3\%$); PCR: 4.26% (95% CI: 2.09%–7.12%); WGS: 1.06% (95% CI: 0.02%–3.16%). Test for subgroup differences: $\chi^2 = 27.27$, $df = 3$, $p < 0.001$. Overall pooled prevalence: 4.64% (95% CI: 2.78%–6.92%).

limited sample size ($n = 1126$). This category was primarily driven by a single study from The Gambia [11] which reported 89 NT-Sp isolates among 121 samples (73.55%), substantially inflating the pooled estimate. Studies focusing exclusively on children showed a pooled prevalence of 4.05% (95% CI: 0.00%–15.80%), while adults showed 3.38% (95% CI: 0.00%–17.99%), and studies, including both children and adults showed 3.06% (95% CI: 0.00%–12.99%). The test for subgroup differences was not statistically significant ($p = 0.659$), indicating that age group categorization alone does not explain the observed heterogeneity. However, the adult subgroup showed no heterogeneity ($I^2 = 0\%$, $p = 0.334$), suggesting consistent NT-Sp carriage estimates across adult study populations. Across included studies, NT-Sp carriage was consistently highest among infants under 24 months of age [8, 11], consistent with the broader epidemiology of pneumococcal carriage where children serve as the primary reservoir for transmission due to immature immune systems and frequent close contact in day-care and household settings.

Vaccination era did not significantly influence NT-Sp carriage ($p = 0.312$), with post-vaccination studies showing 5.72% (95% CI: 2.29%–10.50%) (Figure 5) compared to pre-vaccination studies at 3.87% (95% CI: 1.89%–6.48%) and mixed-era studies at 2.91% (95% CI: 1.74%–4.37%). The non-significant difference between pre- and post-vaccination periods suggests that PCV introduction has not consistently reduced NT-Sp colonization, unlike its dramatic impact on vaccine serotypes. This

persistence may be explained by several mechanisms. First, ecological replacement—the removal of vaccine serotypes creates a niche that NT-Sp (and other non-vaccine serotypes) can fill, as documented in The Gambia where Usuf et al. [11] reported that NT-Sp carriage remained stable despite 10 years of PCV pressure while vaccine serotypes nearly disappeared. Second, capsule loss—some NT-Sp strains represent encapsulated serotypes that have lost capsule expression through genetic mutations in the capsule locus; Sá-Leão et al. [12] identified NT-Sp isolates in Portugal with disrupted capsule loci but otherwise identical genetic backgrounds to serotype 14 strains, suggesting capsule loss as a mechanism of vaccine escape. Third, intrinsic colonization fitness—NT-Sp strains may possess genetic features that enhance mucosal colonization independent of capsule, including frequent absence of the *piaB* gene and presence of *zmpC* [7, 32]. However, studies from Norway [33] and the UK [41] observed declining or stable low NT-Sp carriage post-PCV, indicating that ecological replacement is not universal and may depend on baseline carriage prevalence, population structure, and transmission dynamics. While NT-Sp is rarely implicated in invasive disease, its presence in vaccinated communities underscores its adaptability and the potential for immune evasion.

The high antimicrobial resistance rates observed in NT-Sp isolates are concerning: co-trimoxazole (67.21%, 95% CI: 49.92%–82.73%), penicillin (44.57%, 95% CI: 24.45%–65.50%), and erythromycin (39.85%, 95% CI: 19.58%–61.77%) resistance

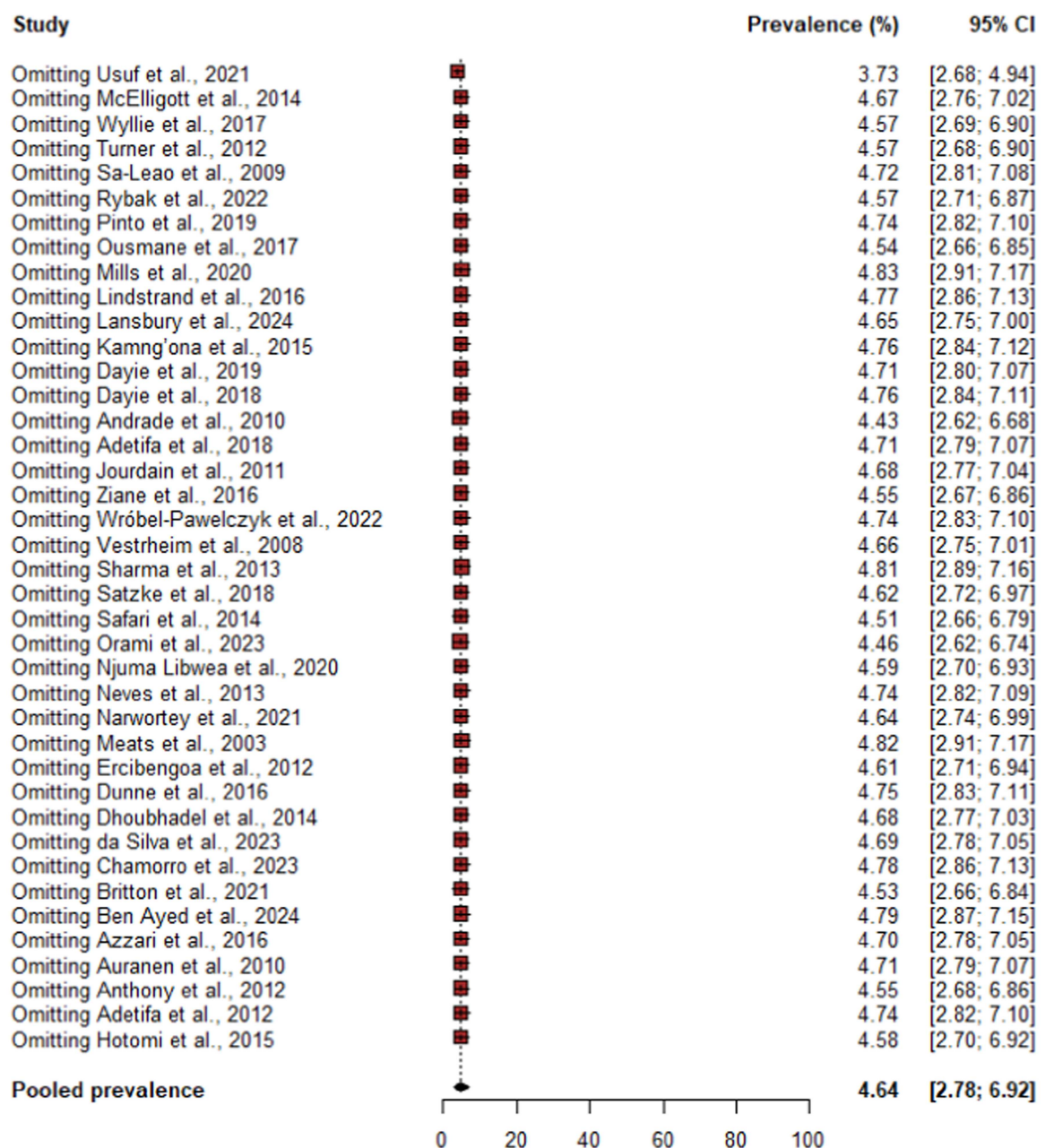


FIGURE 8 | Sensitivity analysis plot showing the effect of removing each study one at a time on the overall pooled estimate. Leave-one-out estimates ranged from 3.73% to 4.83%, all within the 95% CI of the original estimate (4.64%, 95% CI: 2.78%–6.92%). The consistency across iterations confirms that the meta-analysis result is robust and not disproportionately influenced by any single study.

far exceeds typical rates in encapsulated pneumococci in many settings (Figure 9). The overall pooled resistance across all antimicrobials was 46.05% (95% CI: 35.97%–56.26%, $I^2 = 80.0\%$). Several mechanisms may explain why NT-Sp strains harbor resistance at rates comparable to or higher than encapsulated strains. First, the lack of a capsule may reduce the fitness cost associated with carrying resistance genes, allowing NT-Sp to maintain resistance determinants even without antibiotic selection pressure; in encapsulated strains, resistance-conferring mutations or plasmids often impose metabolic costs, and the absence of capsule biosynthesis may spare metabolic resources, enabling NT-Sp to carry multiple resistance genes more stably. Second, co-selection plays a role: NT-Sp strains frequently carry resistance genes (e.g., *ermB* and *tetM*) on the

same mobile genetic elements (such as Tn916-like transposons), as documented by Dayie et al. [26] in Ghana, meaning that exposure to one antibiotic (e.g., tetracycline) could maintain resistance to another (e.g., erythromycin). Third, the high recombination rate of NT-Sp may facilitate acquisition of resistance cassettes from other streptococci (e.g., *Streptococcus mitis* and *S. oralis*) that share the nasopharyngeal niche, with concerning implications for horizontal gene transfer to encapsulated strains that cause invasive disease. Fourth, persistent NT-Sp carriage—potentially longer duration than encapsulated strains in some settings—provides a greater window for antibiotic exposure and resistance selection. Similar phenomena have been observed in non-typeable *Haemophilus influenzae*, suggesting a broader pattern among non-encapsulated

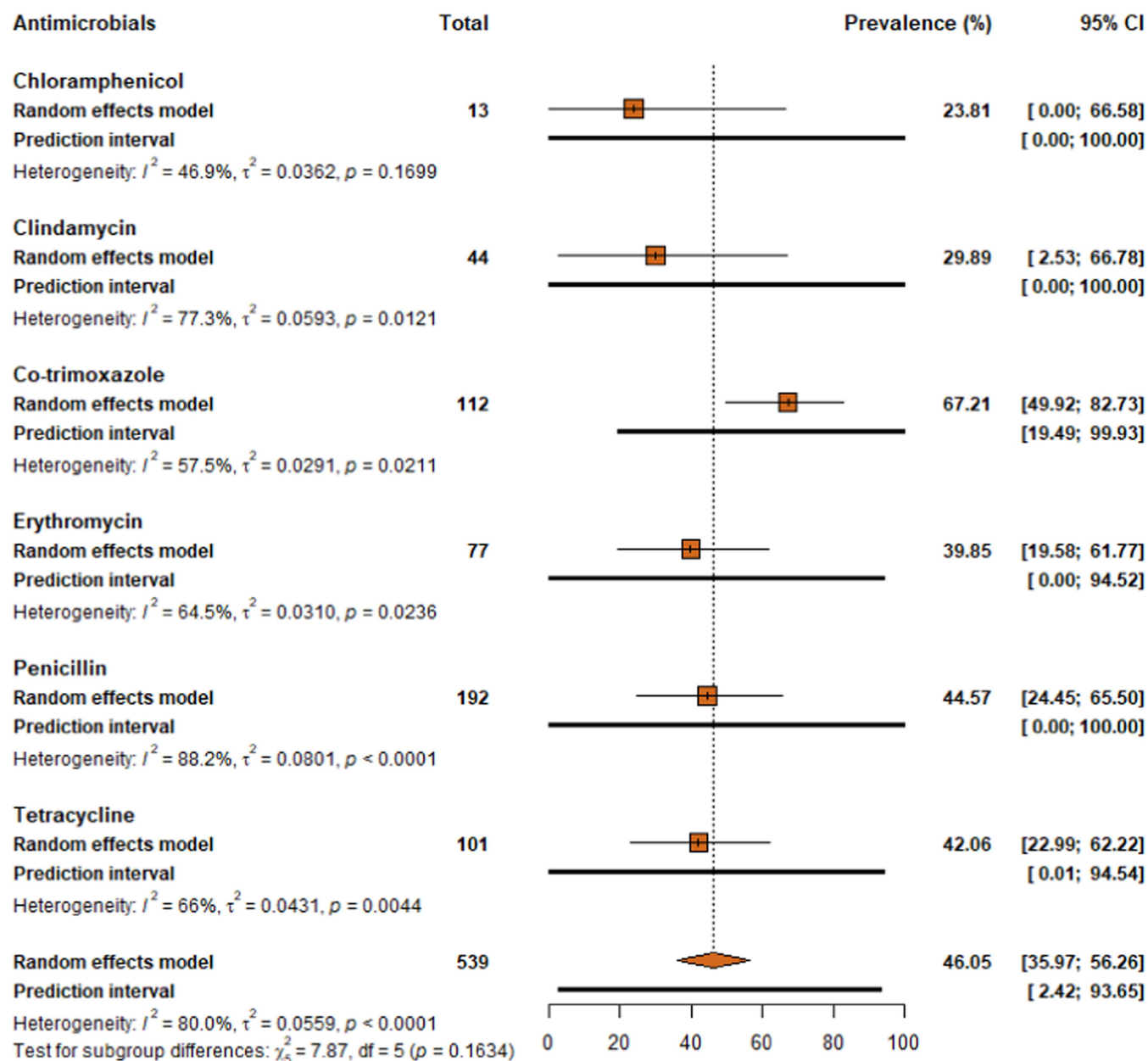


FIGURE 9 | Pooled prevalence of antimicrobial resistance in non-typeable *Streptococcus pneumoniae*. Forest plot showing resistance to six commonly tested antimicrobials. Co-trimoxazole: 67.21% (95% CI: 49.92%–82.73%, $I^2 = 57.5\%$); Penicillin: 44.57% (95% CI: 24.45%–65.50%, $I^2 = 88.2\%$); Tetracycline: 42.06% (95% CI: 22.99%–62.22%, $I^2 = 66.0\%$); Erythromycin: 39.85% (95% CI: 19.58%–61.77%, $I^2 = 64.5\%$); Clindamycin: 29.89% (95% CI: 2.53%–66.78%, $I^2 = 77.3\%$); and Chloramphenicol: 23.81% (95% CI: 0.00%–66.58%, $I^2 = 46.9\%$). Overall pooled resistance: 46.05% (95% CI: 35.97%–56.26%, $I^2 = 80.0\%$). Test for subgroup differences: $\chi^2 = 7.87$, $df = 5$, $p = 0.163$ (not significant).

respiratory pathobionts where capsule loss allows persistence in the face of vaccine pressure but may also facilitate resistance acquisition. The high co-trimoxazole resistance (67%) is particularly concerning because co-trimoxazole remains widely used for pediatric respiratory infections in many low-resource settings, suggesting that empirical co-trimoxazole may be ineffective for pneumococcal infections in these regions, necessitating alternative antibiotics or diagnostic confirmation.

Detection method significantly influenced reported NT-Sp carriage ($p < 0.001$), with multiplex PCR detecting the highest pooled prevalence at 9.48% (95% CI: 7.35%–11.85%), followed by multiple detection tools at 4.67% (95% CI: 2.69%–7.14%,

$I^2 = 97.3\%$), PCR at 4.26% (95% CI: 2.09%–7.12%), and WGS at 1.06% (95% CI: 0.02%–3.16%) (Figure 7). The substantially lower prevalence detected by WGS likely reflects more stringent confirmation of pneumococcal identity and exclusion of non-pneumococcal isolates, particularly among viridans group streptococci such as *Streptococcus mitis* that can be misidentified as pneumococci by phenotypic methods. This finding underscores the importance of standardized molecular confirmation for accurate NT-Sp surveillance and highlights that many older studies relying on Quellung reaction alone may have significantly underestimated NT-Sp prevalence. Studies using enrichment culture plus PCR consistently detected higher NT-Sp carriage than those relying on Quellung reaction alone,

reinforcing that detection methods are a critical determinant of reported prevalence and that molecular approaches should be prioritized, especially in low-resource settings.

Meta-regression analysis identified participant category “All ages” as the only statistically significant predictor of NT-Sp carriage prevalence (coefficient: 0.4776, 95% CI: 0.0360–0.9193, $p = 0.034$) (Table 4). Studies that included participants of all ages reported significantly lower NT-Sp prevalence compared to studies focusing exclusively on children (the reference category). This finding is consistent with the age-stratified subgroup analysis, which showed that children have the highest NT-Sp carriage rates, and including adults in study populations dilutes the overall prevalence estimate. No other covariates reached statistical significance, though several trends were observed: Asian studies showed a trend toward higher prevalence (coefficient: 0.0957, $p = 0.260$), North American studies showed a trend toward lower prevalence (coefficient: -0.0766 , $p = 0.648$), multiplex PCR detection showed a trend toward higher prevalence (coefficient: 0.1660, $p = 0.322$), and WGS detection showed a trend toward lower prevalence (coefficient: -0.0336 , $p = 0.844$). Vaccination period showed no association with NT-Sp prevalence (coefficient: -0.0523 , $p = 0.426$ for pre-vaccination vs. post-vaccination). The lack of statistically significant findings for most covariates underscores the complexity of NT-Sp epidemiology, where unmeasured factors (e.g., host immunity, local antibiotic use, socioeconomic status, and transmission dynamics) likely play critical roles in explaining the substantial heterogeneity observed across studies.

Molecular tools provided key insights into the genetic diversity and evolution of NT-Sp. MLST and WGS confirmed region-specific clones and demonstrated that NT-Sp strains share most of their core genome with encapsulated lineages [26, 28]. Clonal complexes such as ST66 were identified in Brazil [28], and ST320 in Ghana [26], illustrating regional specificity and geographic structure, likely reflecting local transmission dynamics, antibiotic selection pressures, and vaccine introduction histories. In Southeast Asia and parts of Africa, studies noted the presence of non-encapsulated

clones lacking traditional capsule biosynthesis genes but retaining key virulence and resistance determinants. Genomic analyses demonstrated that NT-Sp strains shared up to 90.6% core genome similarity with encapsulated strains, though lacking capsule synthesis loci. The absence of the *cps* locus (capsule biosynthesis genes) is the defining molecular feature of NT-Sp, but other genetic markers distinguish NT-Sp from encapsulated strains; the frequent presence of *zmpC* (zinc metalloprotease C, associated with mucosal adhesion) and absence of *piaB* (pneumococcal iron acquisition B) in NT-Sp isolates [32] suggest that NT-Sp has adapted to the mucosal environment through loss of capsule but retention of colonization factors. Accessory gene content, including prophages, surface proteins, and AMR genes varied significantly, reflecting adaptation to specific ecological niches. Some NT-Sp strains carried resistance determinants such as *mefA*, *ermB*, and *tetM*, further underscoring their role in antimicrobial resistance gene dissemination. The emergence of NT-Sp may reflect ongoing pneumococcal evolutionary adaptation through capsule switching, capsule loss, and recombination-mediated genome plasticity under vaccine and antibiotic selective pressure. Standardization remains a challenge, as older serotyping methods frequently misclassified NT-Sp, and the review highlights the need for consistent application of WGS and MLST, especially in low-resource settings, to ensure accurate surveillance. The high genomic similarity between NT-Sp and encapsulated strains suggests that NT-Sp may serve as a “genetic reservoir” that can exchange genes with encapsulated strains, potentially transferring resistance determinants, virulence factors, or even capsule loci, with implications for vaccine escape and resistance dissemination.

Despite these findings, many studies relied on older phenotypic assays, underestimating NT-Sp prevalence. Detection tools significantly influenced reported outcomes: studies using modern PCR and WGS methods consistently detected NT-Sp even when earlier surveys had not. Standardizing detection protocols and increasing the use of molecular tools especially in Africa and Southeast Asia, will be essential for future studies. Importantly, detection methods influenced reported prevalence: studies

TABLE 4 | Meta-regression examining the effect of covariates on NT-Sp carriage prevalence.

Coefficient	Estimate	SE	z-value	p-value	95% CI lower	95% CI upper	Significance
Intercept	0.1132	0.1862	0.6077	0.5434	−0.2518	0.4782	
Continent – Asia	0.0957	0.0849	1.1264	0.2600	−0.0708	0.2622	
Continent – Europe	0.0263	0.0762	0.3450	0.7301	−0.1230	0.1755	
Continent – North America	−0.0766	0.1677	−0.4565	0.6480	−0.4052	0.2521	
Continent – Oceania	0.0736	0.1043	0.7053	0.4806	−0.1309*	0.2780	
Continent – South America	0.0603	0.1042	0.5785	0.5630	−0.1439	0.2645	
Participant – All ages	0.4776	0.2253	2.1196	0.0340	0.0360	0.9193	*
Participant – Children	0.0871	0.1875	0.4646	0.6422	−0.2804	0.4546	
Participant – Children and adults	0.0532	0.1863	0.2856	0.7752	−0.3119	0.4183	
Detection – Multiplex PCR	0.1660	0.1677	0.9898	0.3223	−0.1627	0.4948	
Detection – PCR	0.0728	0.2403	0.3031	0.7618	−0.3981	0.5437	
Detection – WGS	−0.0336	0.1705	−0.1973	0.8436	−0.3678	0.3005	
Vaccination – pre- and post-vaccination	−0.0388	0.0940	−0.4129	0.6797	−0.2230	0.1454	
Vaccination – pre-vaccination	−0.0523	0.0656	−0.7967	0.4256	−0.1808	0.0763	

*Statistically significant at $p < 0.05$. Bold values indicate statistically significant covariates

using enrichment culture plus PCR consistently detected higher NT-Sp carriage than those relying on Quellung reaction alone. No consistent differences were observed by HIV status: Safari et al. [36] detected NT-Sp in HIV-infected Indonesian children, while Anthony et al. [51] found no significant association in Tanzanian HIV-positive children. Among children with sickle cell disease in Ghana, Dayie et al. [27] reported low NT-Sp carriage but noted high antimicrobial resistance. Rural settings in The Gambia [11] showed higher carriage than urban areas, suggesting transmission intensity as a key driver. Clinically, NT-Sp was most frequently associated with conjunctivitis and otitis media [17], with rare invasive disease.

4.1 | Limitation

Substantial heterogeneity was observed across all meta-analyses (I^2 consistently > 90%), reflecting variation in study designs, sampling strategies, laboratory methods, and population characteristics that could not be fully explained by subgroup analyses or meta-regression.

NT-Sp identification methods varied considerably across studies, ranging from Quellung reaction to whole-genome sequencing. Detection method subgroup analysis confirmed that multiplex PCR detected significantly higher prevalence (9.48%) compared to WGS (1.06%), suggesting possible misclassification and underestimation in studies relying solely on phenotypic methods.

Antimicrobial resistance data were inconsistently reported, with most studies using phenotypic assays rather than molecular confirmation of resistance genes. Heterogeneity in resistance estimates was substantial (I^2 range: 46.9%–88.2%).

Selection bias may have been introduced as only open-access and free full-text articles published in English were included.

NT-Sp remains underrepresented in invasive disease surveillance, limiting understanding of its role in horizontal gene transfer and AMR gene dissemination.

Risk of bias assessment using the Newcastle–Ottawa Scale revealed that six studies (15%) were rated as moderate quality. However, sensitivity analysis (Figure 8) confirmed the robustness of the overall findings (leave-one-out estimates: 3.73%–4.83%).

The “All ages” subgroup estimate (29.88%) should be interpreted with caution as it was primarily driven by a single study with exceptionally high NT-Sp carriage.

Publication bias was not evident (Egger’s test: $p = 0.8212$) (Figure 6), and sensitivity analyses (Figure 8) supported the robustness of our conclusions.

5 | Conclusion

This review reaffirms that non-typeable *Streptococcus pneumoniae* (NT-Sp) is a globally distributed and genetically diverse

subset of pneumococci that deserves greater attention in the post-vaccine era. Despite their historically overlooked status, NT-Sp strains demonstrate significant carriage prevalence, high levels of antimicrobial resistance, and considerable genetic adaptability across diverse settings and populations.

The persistence of NT-Sp in both vaccinated and unvaccinated populations, alongside its resistance to multiple antibiotics, positions it as a potentially important reservoir of antimicrobial resistance determinants within pneumococcal populations. Furthermore, the detection of region-specific clones and the high genomic similarity to encapsulated strains suggest that NT-Sp may play an important evolutionary role in pneumococcal ecology.

To address these concerns, global pneumococcal surveillance efforts must routinely include NT-Sp, applying modern molecular tools such as WGS and MLST. These tools not only improve detection but also provide essential data on strain diversity, resistance mechanisms, and potential for vaccine escape. In addition, future vaccine research could explore whether broadly protective protein antigens (e.g., PspA and pneumolysin) currently under development would also cover NT-Sp, given their high genomic similarity to encapsulated strains. However, the present review did not directly evaluate vaccine candidates, and this remains a hypothetical direction requiring further pre-clinical investigation.

Based on these findings, the following actions are recommended. For clinical practice: (1) in settings where NT-Sp carriage prevalence exceeds 10%, laboratories should add molecular confirmation (cpsA-PCR) for optochin-sensitive, non-Quellung-reactive isolates; (2) empirical antibiotic therapy for recurrent otitis media or suspected pneumococcal carriage-related infections in high-NT-Sp areas should account for local resistance patterns, particularly the high prevalence of co-trimoxazole resistance (67%). For research: (3) future carriage surveillance studies should routinely report NT-Sp separately and use standardized definitions (cpsA-negative and lytA-positive); (4) WGS should be prioritized in low-resource settings to track NT-Sp clones and resistance gene dissemination; (5) longitudinal studies are needed to determine whether NT-Sp carriage precedes or predicts acquisition of resistant encapsulated strains.

In summary, NT-Sp is not merely a passive bystander in pneumococcal biology. It is a dynamic and clinically relevant population that requires targeted research, routine monitoring, and strategic inclusion in both antimicrobial and vaccine policy.

Author Contributions

Robert K. Reeks: conceptualization, investigation, funding acquisition, writing – original draft, writing – review and editing, visualization, methodology, software, formal analysis, project administration, data curation. **Onyansaniba K. Ntim:** writing – review and editing, writing – original draft. **Eric S. Donkor:** funding acquisition, writing – review and editing, supervision, resources.

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Disclosure

All authors have read and approved the final version of the manuscript. Eric S. Donkor (corresponding author) had full access to all of the data in this study and takes complete responsibility for the integrity of the data and the accuracy of the data analysis.

Ethics Statement

The authors have nothing to report.

Consent

The authors have nothing to report.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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