Research Square

Preprints are preliminary reports that have not undergone peer review.
They should not be considered conclusive, used to inform clinical practice,
or referenced by the media as validated information.

Resistance to Second-Line Antibiotics in *Mycoplasma pneumoniae* Isolates From Children in East Asia: A Systematic Review and Meta-analysis

Hao Chen
Beijing Children's Hospital
Deze Li
Beijing Children's Hospital
Chen Shen
shenchen1110@126.com
Beijing Children's Hospital https://orcid.org/0000-0002-7806-5723

Systematic Review

Keywords: Mycoplasma pneumoniae, children, tetracyclines, fluoroquinolones, antimicrobial resistance, meta-analysis

Posted Date: November 19th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-11747171/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License. Read Full License

Additional Declarations: The authors declare no competing interests.

Abstract

Background: Macrolide-resistant *Mycoplasma pneumoniae* (MRMP) is a therapeutic challenge in children, especially in East Asia. Tetracyclines and fluoroquinolones are alternatives when macrolides fail, but their *in vitro* resistance rates have not been synthesized by susceptibility-testing method.

Methods: A unified search, with Chinese and English strings as block-for-block mirror images, covered six databases to September 1, 2026. Of 1,136 records, 700 remained after deduplication. Twenty pediatric studies were eligible; eleven tested cultured isolates by standardized dilution and formed the primary analysis of all isolates regardless of macrolide-resistance status, one contributed to sensitivity analysis, and eight were descriptive. Because all primary analyses observed zero events, results are pooled as 0.00% with exact upper 95% bounds.

Results: No resistant isolate was observed for any second-line agent by each study's own criteria. Pooled resistance was 0.00% for tetracycline (n=2,353; upper bound 0.16%), levofloxacin (n=2,175; 0.17%), minocycline (n=1,545; 0.24%), moxifloxacin (n=805; 0.46%), and doxycycline (n=608; 0.60%); resistance was zero for both all-isolate and macrolide-resistant denominators. Interpretive criteria differed; a sensitivity analysis limited to CLSI M43-A studies gave the same result. One serology-based study without cultured isolates was not pooled.

Conclusions: Across China and Japan (2002-2025), no cultured isolate resistant to tetracyclines or fluoroquinolones was observed. Absence of observed resistance is not evidence of absence: upper bounds (0.16%-0.60%) remain compatible with low-frequency resistance. Macrolides remain first-line; second-line agents retain activity, but standardized surveillance is needed.

Introduction

Introduction *Mycoplasma pneumoniae* is a major cause of community-acquired pneumonia in school-aged children and adolescents worldwide [1]. Because the organism lacks a cell wall, beta-lactam antibiotics have no direct activity, and macrolides have historically been used as first-line therapy in children. The clinical problem has become more complex as macrolide-resistant *M. pneumoniae* (MRMP) has emerged and persisted, particularly in East Asia [2,3]. Global surveillance syntheses have shown substantial regional variation in the proportion of MRMP, with markedly higher proportions reported in several Asian settings than in many European or North American cohorts [4]. In clinical practice, MRMP is important because microbiological resistance may be associated with delayed defervescence, prolonged symptoms, and uncertainty about when to change therapy, especially during large outbreaks or when molecular resistance testing is not rapidly available [5,6]. Recent epidemics have therefore renewed interest in effective alternatives when macrolide therapy fails [7]. Tetracyclines and fluoroquinolones are the principal second-line drug classes with activity against *M. pneumoniae*. Their mechanisms differ from those of macrolides: macrolide resistance is usually linked to mutations in domain V of the 23S rRNA gene, whereas tetracyclines act through inhibition of bacterial protein synthesis at the 30S ribosomal subunit and fluoroquinolones act through inhibition of DNA gyrase and topoisomerase IV [1,6]. This mechanistic separation makes direct cross-resistance biologically unlikely, but it does not eliminate the need for surveillance. Increased use of second-line agents during MRMP outbreaks could create new selection pressure, and resistance to these agents would substantially narrow treatment options in children. Pediatric use of second-line agents also requires caution. Doxycycline and related tetracyclines may be considered in older children or in selected severe infections, but age, duration of therapy, local guidance, and risk-benefit balance remain central considerations [6,8]. Fluoroquinolones such as levofloxacin and moxifloxacin have activity against *M. pneumoniae*, but pediatric use is generally reserved for situations in which benefits outweigh potential safety concerns, including musculoskeletal adverse effects and broader antimicrobial stewardship considerations [6,8]. For these reasons, *in vitro* susceptibility data cannot be translated directly into treatment recommendations; they should instead be interpreted alongside clinical severity, age, prior treatment response, and local prescribing guidance. The primary objective of this systematic review and meta-analysis was to estimate drug-specific *in vitro* resistance to tetracyclines and fluoroquinolones among pediatric MRMP isolates in East Asia, with separate treatment of studies using reference or equivalent dilution methods and studies using non-standardized methods. Secondary objectives were to describe between-study heterogeneity, assess the limitations of sparse zero-event data, and identify evidence gaps relevant to standardized pediatric MRMP surveillance.

Materials and Methods

2.1 Protocol and Registration

This systematic review and meta-analysis was conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) 2020 statement [9]. The protocol was registered prospectively with PROSPERO (CRD420261411817). The completed PRISMA 2020 checklist is provided in Supplementary Table 1. The registered protocol specified macrolide-resistant (MRMP) isolates as the primary-analysis denominator. Before data extraction, the primary analysis was amended to include all cultured pediatric *M. pneumoniae* isolates irrespective of macrolide-resistance status, because resistance to second-line agents (tetracyclines: ribosomal protection and efflux; fluoroquinolones: *gyrA* and *parC* mutations) is genetically independent of macrolide resistance (23S rRNA domain V mutation). This amendment was motivated by resistance biology rather than by observed results, macrolide-resistance status was retained as a stratifying variable in a secondary analysis, and a secondary analysis restricted to MRMP isolates yielded the same zero-event finding.

2.2 Search Strategy

A single, unified search strategy was applied across all databases, using one concept framework with four Boolean blocks combined with AND: (1) *Mycoplasma pneumoniae*; (2) second-line agents (tetracyclines and fluoroquinolones); (3) resistance or susceptibility; and (4) the pediatric population. The Chinese-language and English-language search strings were constructed as direct mirror images of one another, block for block, so that the two tracks differ

only in language and database syntax. All databases were searched to a common cut-off date of September 1, 2026, and eligible reports were restricted to English and Chinese. To ensure reproducibility and a consistent cut-off across platforms, PubMed was searched with the same simplified block strategy used in Web of Science and Scopus; the subscription databases (Web of Science, Scopus) were searched by an institutional operator with access, and Embase was prepared but could not be searched because institutional access was not available.

Two Chinese-language databases and four English/international databases were searched, with a fifth (Embase) prepared but not run. The Chinese track comprised CNKI (n = 191) and WanFang Data (n = 241); VIP and SinoMed/CBM were not searched. The English/international track comprised PubMed (n = 151), Web of Science Core Collection (n = 144), Scopus (n = 399), and the Cochrane Central Register of Controlled Trials (CENTRAL; n = 10), all to the September 1, 2026 cut-off. Embase could not be searched because institutional access was not available; the prepared Embase strategy is retained in Supplementary Table 2 for reproducibility and future updates, and this constraint is acknowledged as a limitation.

In total, 1,136 records were identified (Chinese track 432: CNKI 191, WanFang 241; English/international track 704: PubMed 151, Web of Science 144, Scopus 399, CENTRAL 10). Duplicates were removed by normalized title-author-year matching within and across databases. In the Chinese track, the two databases overlapped heavily (432 to 254 unique records). In the English track, cross-database indexing duplicates were removed (704 to 446 unique records). Because Chinese-language and English-language reports seldom describe the same study, the two tracks were additive, giving 700 unique records for title and abstract screening. The complete, verbatim search strategy for every database, Chinese and English, is provided in Supplementary Table 2. All 10 CENTRAL records were trial registrations or clinical-efficacy studies, and none was eligible.

2.3 Eligibility Criteria

Studies were eligible if they (1) cultured and identified *Mycoplasma pneumoniae* isolates (infection diagnosed serologically or by nucleic acid amplification alone, without a cultured isolate, was not eligible); (2) performed *in vitro* susceptibility testing of at least one tetracycline or fluoroquinolone by a dilution method with defined interpretive criteria; (3) included a pediatric population (≤ 18 years) or a separately reported pediatric subgroup; (4) described the testing method and reference standard; and (5) reported isolate-level minimum inhibitory concentrations (MICs) or categorical interpretations from which drug-specific resistant counts could be obtained. Reviews, case reports, studies reporting only aggregate MIC50/MIC90 summaries without isolate-level counts, and studies using methods without *M. pneumoniae* breakpoints (for example, disc diffusion) were excluded from the quantitative analysis.

The primary analysis estimated second-line resistance among all cultured pediatric *M. pneumoniae* isolates, irrespective of macrolide-resistance status, because resistance to tetracyclines (ribosomal protection and efflux) and to fluoroquinolones (*gyrA* and *parC* quinolone-resistance-determining-region mutations) arises through mechanisms genetically independent of macrolide resistance (23S rRNA domain V mutation). The denominator for each drug in each study was the number of isolates actually tested against that drug, whether macrolide-resistant or macrolide-susceptible; where a study tested only its macrolide-resistant subset, that subset was the denominator. Macrolide-resistance status was retained as a stratifying variable rather than an inclusion threshold: a secondary analysis restricted the denominator to macrolide-resistant (MRMP) isolates, and, where a study separately reported macrolide-susceptible isolates, a macrolide-resistant versus macrolide-susceptible comparison was tabulated. MRMP status was accepted as defined and reported by each study, because macrolide resistance thresholds differ between agents (for example, erythromycin and azithromycin) and are not standardized for *M. pneumoniae*.

When two reports described overlapping isolate collections from the same center, surveillance network, or research group, we applied a tiered policy: (i) reports fully subsumed by a more comprehensive report of the same collection were excluded as duplicates; (ii) reports with a confirmed partial temporal overlap whose overlapping isolates could not be separated were excluded from the primary analysis but could enter sensitivity analyses; (iii) reports with only a small, unconfirmed potential overlap with an included independent collection were retained, with leave-one-out sensitivity analysis.

2.4 Study Selection

Two reviewers independently screened titles and abstracts and then assessed potentially eligible full texts. Disagreements were resolved by discussion, with adjudication by a third reviewer when required. To manage the volume of Chinese-language records, title and abstract screening of that track was supported by a large language model, which classified each record as potentially eligible or clearly ineligible against the pre-specified criteria; every record the model marked potentially eligible, and a verification sample of those it marked ineligible, were confirmed by a human reviewer, and all studies ultimately included were verified against their full text by the reviewers. The language model was used only as a screening aid and made no final inclusion, data-extraction, or interpretation decisions. The selection process is shown in Fig. 1.

2.5 Data Extraction

A standardized data extraction form was developed and pilot-tested. Extracted data included: first author, publication year, study period, geographic location, study design, sample size, patient age range, antimicrobial susceptibility testing method, reference standard, tested antibiotics, resistance rates (with numerators and denominators), intermediate rates, and genetic characterization data. Data extraction was performed independently by two reviewers, with cross-verification for accuracy. When studies reported different numbers of isolates tested for different antibiotics, the drug-specific denominator was recorded and used for each respective meta-analysis. Consequently, the total sample size (n) varies across drug-specific analyses within the same study. During revision, all extracted data were re-verified against the original publications, and study-level corrections are documented in Supplementary Table 6.

2.6 Quality Assessment

Methodological quality was assessed with the Joanna Briggs Institute (JBI) Critical Appraisal Checklist for Studies Reporting Prevalence Data, a validated nine-item instrument appropriate for studies that report a proportion. Each item was rated yes, no, or unclear. Because resistance was treated as a proportion, the two items framed for population surveys (sampling frame and response rate) were interpreted as the representativeness and completeness of the isolate denominator. Studies were classified as low, moderate, or high risk of bias, with the validity and standardization of the susceptibility method

(items 6 and 7: a valid method for identifying the condition, and consistent measurement against defined criteria) treated as decisive, because an unstandardized or inapplicable susceptibility method undermines the resistance estimate regardless of other strengths. This classification determined the analysis stratum: studies using culture-confirmed isolates with broth microdilution and defined breakpoints were eligible for the primary analysis, whereas studies using non-standardized methods, commercial kits without CLSI concordance, or serological diagnosis without isolates were confined to the descriptive analysis. Appraisal drew on the full text of all twenty studies; per-item ratings are provided in Supplementary Table 3. Ratings were used descriptively and were not converted into a numerical weight. The overall strength of the body of evidence was summarized with a structured framework adapted for prevalence and surveillance data rather than with GRADE, because GRADE is designed primarily for intervention-effect questions and does not have an established procedure for non-comparative, zero-event prevalence estimates. The summary integrated the JBI critical-appraisal results with four further dimensions, methodological design, sample size and statistical precision, consistency and representativeness across settings and periods, and breakpoint comparability; the resulting evidence-quality summary is presented in Supplementary Table 4.

2.7 Statistical Analysis

Drug-specific resistance rates were synthesized across studies. Because every primary-analysis study reported zero resistant isolates for the drugs evaluated, conventional meta-analytic heterogeneity cannot be assessed and random-effects pooling of proportions is uninformative. We therefore report the pooled resistance rate as 0.00% together with the exact (Clopper-Pearson) 95% confidence upper bound computed on the combined numerator and denominator of each drug-specific analysis. This bound is the upper limit of the resistance rate that is statistically compatible with observing zero events across the combined sample; it is the pooled-sample analogue of the rule of three for a single proportion. Each drug-specific bound was computed independently and reflects inference for that agent alone; no adjustment for multiple comparisons was applied, so the bounds should be read as per-drug limits rather than a simultaneous family-wise statement. The approach does not assume homogeneity across studies: zero events provide no empirical evidence of between-study variation, so the pooled bound is a conservative statement about the true resistance rate given the combined data, rather than a random-effects summary. Two kinds of sensitivity analysis were pre-specified and are distinguished throughout. The first is a study-level sensitivity analysis: Jiang Y 2024, which did not meet the primary-analysis denominator requirement (its pediatric MRMP subset could not be separated from a mixed pediatric and adult specimen pool), was added to the primary-analysis pool to test whether including this methodologically less restrictive study altered the drug-specific bounds. The second is a statistical sensitivity analysis: random-effects models (DerSimonian-Laird) with a 0.5 continuity correction for zero-event studies were fitted as a secondary check on whether non-zero point estimates emerge when zero-event studies are given a small pseudocount. Both are reported in Supplementary Table 8, and neither materially altered the conclusions. Because CLSI provides no resistance breakpoints for tetracyclines or fluoroquinolones against *M. pneumoniae*, isolate-level resistance classifications were adopted as reported by each study under its stated interpretive criteria: where only a susceptible-only cutoff existed (levofloxacin), isolates above that cutoff were reported narratively as non-susceptible and were not counted as resistant; where no breakpoint existed at all (minocycline in Wang N 2022, Wu Q 2026, and Wang Y 2016), results were reported as MIC distributions and not pooled. Consequently, pooled zero-event rates denote zero isolates classifiable as resistant under study-level criteria, and pooled denominators conservatively retain non-susceptible isolates that could not be classified. Heterogeneity was summarized with Cochran's Q and the I^2 statistic where estimable; we note that heterogeneity statistics are uninformative when all contributing studies report zero events. Analyses with only one study were reported descriptively. Three sensitivity analyses were performed: (i) adding Jiang Y 2024, whose MIC-tested isolate subset was not age-stratified, to the tetracycline and levofloxacin analyses; (ii) excluding Yan C 2025, because its Beijing subset (24 isolates) may overlap with the 2017–2025 Beijing collection of Yan C 2026; and (iii) adding Akaike H 2012, which was excluded as an overlapping cohort with Oishi T 2019, to the tetracycline and minocycline analyses. Because all primary analyses included no more than nine studies and observed zero events, formal assessment of publication bias (funnel plots, Egger's test, and Begg's test) was not performed, consistent with methodological guidance that such tests are unreliable when few studies are available [10, 11]. Two-sided P values < 0.05 were considered statistically significant. All statistical analyses were performed using R version 4.4.1 (R Foundation for Statistical Computing, Vienna, Austria); meta-analyses of single proportions were conducted with the meta package.

Results

3.1 Study Selection and Characteristics

A single, unified search, with Chinese and English strings built as block-for-block mirror images and a common cut-off of September 1, 2026, identified 1,136 records in two tracks: Chinese databases (CNKI 191; WanFang 241; subtotal 432) and English/international databases (PubMed 151, Web of Science 144, Scopus 399, CENTRAL 10; subtotal 704). The two Chinese databases overlapped heavily and deduplicated to 254 unique records; the English databases deduplicated to 446 unique records after removal of cross-database indexing duplicates. Because Chinese-language and English-language reports seldom describe the same study, the tracks were additive, yielding 700 unique records for title and abstract screening. Title and abstract screening retained 113 records for full-text assessment (Chinese 83; English 30); full-text review against the eligibility criteria then excluded 93 reports, the main reasons being the absence of extractable isolate-level second-line susceptibility data, treatment- or efficacy-only designs without susceptibility testing, macrolide-only testing, reviews or case reports, and molecular or diagnostic studies without second-line results (Fig. 1). Twenty pediatric studies met the eligibility criteria for qualitative synthesis, of which eleven met the additional requirements for the primary analysis, studies using standardized dilution methods in pediatric MRMP or predominantly MRMP isolates [12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22]; one study was retained for sensitivity analyses only [7]; and eight studies using non-standardized methods, non-isolate-based denominators, or summary-level MIC reporting were summarized separately as descriptive evidence [23, 24, 25, 26, 27, 28, 29, 42]. All 20 included studies were identified through the systematic search; 11 entered the quantitative (pooled) analysis, 8 the descriptive analysis, and 1 the sensitivity analysis only. Per-report screening decisions and exclusion reasons for all 87 original-search full-text reports are provided in Supplementary Table 7; decisions for the 26 updated-search reports are summarized in Supplementary Table 6. During revision, all data were re-extracted against the original publications, and two classification changes were made. He WQ 2025 was reclassified from the primary to the descriptive analysis because *M. pneumoniae* infection was diagnosed serologically (passive agglutination, antibody titer ≥ 1:160) without cultured isolates or MRMP genotyping, and drug testing used a "serial dilution" method without a CLSI standard, interpretive breakpoints, or a quality-control strain. Jiang Y 2024 was

restricted to sensitivity analyses because its denominator of 4,145 comprised respiratory specimens (3,916 children and 229 adults), only 45 of the 481 cultured isolates underwent MIC testing, and that subset was not age-stratified, so a pediatric MRMP denominator could not be extracted. The updated search contributed eight additional primary-analysis studies: Morozumi M 2005 (195 pediatric isolates, Japan, 2002–2004; MRMP subset n = 12; PPLO broth microdilution), Oishi T 2019 (1,256 pediatric isolates from 85 Japanese institutions, 2011–2016; MRMP n = 873; PPLO broth microdilution), Guo D 2019 (65 isolates from 11 Beijing hospitals, 2014; MRMP n = 57; broth microdilution with author-adapted CLSI M100-S20 breakpoints), Wang Y 2016 (18 macrolide-resistant isolates from a school outbreak in Hangzhou, 2014; CLSI M43-P broth dilution), Liu Y 2010 (100 pediatric isolates from Shanghai Children's Hospital, March 2008-July 2009; MRMP n = 90; SP4 broth microdilution by the Waites 2001 method with FH reference control), Yan C 2026 (197 pediatric MRMP isolates from Beijing, 2017–2025; CLSI M43-A broth microdilution), Wu Q 2026 (148 pediatric MRMP isolates from Shanghai, 2023–2024, of which 146 underwent susceptibility testing; CLSI M43-A broth microdilution), and Fang M 2026 (227 pediatric MRMP isolates from Jinan, 2021–2024; CLSI M43-A broth microdilution). The updated search also prompted full-text exclusions with reasons. Four reports were excluded as overlapping cohorts, retaining the most comprehensive report in each case: Jia X 2024 [30], originating from the same center and research group as Yan C 2026 (Capital Institute of Pediatrics, Beijing) with yearly isolate counts for 2021 (n = 13) and 2022 (n = 18) identical to the corresponding strata of Yan C 2026, was excluded post hoc as subsumed by the more comprehensive report; Akaike H 2012 [31] (Atypical Pathogen Study Group [APSG] surveillance, November 2009-August 2011) overlaps with the APSG-based Oishi T 2019 collection (2011–2016) during January-August 2011; Wang N 2020 [32] reported 110 isolates (2017–2018) that are a subset of the 182 isolates in Wang N 2022; and Kawai Y 2013 [33] was excluded because it reported MIC90 summaries only, with no extractable isolate-level data (criterion 5), and its APSG-network coverage is fully represented by the more comprehensive Oishi T 2019. Liu Yang 2009 [34] was excluded because it provides no extractable second-line isolate-level counts; it describes the same Shanghai cohort as the corresponding English-language report, which was likewise ineligible for the same reason. Huang C 2025 [35] was excluded a priori for invalid methodology: susceptibility testing used a commercial colorimetric kit designed for Ureaplasma and *M. hominis*, with a 24–48 h incubation incompatible with slow-growing *M. pneumoniae* and no interpretive breakpoints or MICs; its implausibly high reported resistance (38.6% tetracycline, 31.4% quinolones) is consistent with method invalidity rather than an independent exclusion ground, and the study is discussed below as a cautionary example. Liu Y 2010 [22] (100 pediatric isolates, Shanghai Children's Hospital, March 2008-July 2009; 90 macrolide-resistant isolates), initially left unclassified because the full text was paywalled at the time of the updated search, was included in the primary analysis after the full text was obtained and verified during this revision: susceptibility testing used SP4 broth microdilution (Waites 2001 method) with the FH (ATCC 15531) reference strain in triplicate, and all six second-line agents (tetracycline, doxycycline, minocycline, levofloxacin, moxifloxacin, and ciprofloxacin) showed zero resistance among the 90 MRMP isolates. A further nine updated-search reports were excluded because they lacked extractable isolate-level pediatric culture-based susceptibility data (mixed-age populations without separable pediatric data, molecular-only detection, a single case report, a reference-strain collection, pharmacodynamic or killing-kinetics methods studies [36, 37], or clinical treatment-outcome studies [5, 38]). The twenty included studies are summarized in Table 1. The eleven primary studies comprised eight from China and three from Japan, with isolate collections ranging from 18 (Wang Y 2016, a school-outbreak collection) to 1,256 (Oishi T 2019, an 85-institution national collection), and surveillance spanning 2002–2025 (China, 2008–2025; Japan, 2002–2020). All eleven primary studies used conventional or PPLO broth microdilution read against CLSI M43-A or equivalent reference criteria, whereas the eight descriptive-analysis studies used a serology-based serial dilution method, a commercial kit, an insufficiently described method, a non-CLSI-M43-A historical standard, summary-level MIC reporting without isolate-level resistance counts, or standardized susceptibility testing completed only in a small isolate subset of uncertain representativeness (Shi RH 2026). Study-level methodological assessments are reported in Supplementary Table 3; the adapted checklist was interpreted descriptively. Gene detection methods and key findings are summarized in Table 2. Most primary studies confirmed macrolide resistance by sequencing or PCR detection of 23S rRNA mutations (Wu Q 2026 used whole-genome sequencing; all 148 isolates carried A2063G); Oishi T 2022 defined MRMP phenotypically from the bimodal macrolide MIC distribution and did not perform gene analysis. Testing for fluoroquinolone- or tetracycline-associated resistance determinants was inconsistently reported. The interpretive breakpoints applied in each study are now tabulated in Table 2; notably, CLSI provides no resistance breakpoints for tetracyclines or fluoroquinolones against *M. pneumoniae* [39], the minocycline results of Wang N 2022, Wu Q 2026, and Wang Y 2016 were therefore not classifiable (reported as MIC distributions only), and Guo D 2019 applied author-adapted breakpoints derived from CLSI M100-S20 rather than M43-A.

Table 1
Characteristics of the 20 included studies.

Study	Year	Location	Period	Design	N	MRMP n (%)	Population	Susceptibility method	Risk of bias	Analysis group
Morozumi M 2005	2005	Japan (multi-center)	2002–2004	Cross-sectional	195	12 (6.2%)	Pediatric CAP	PPLO broth microdilution (QC strain M129)	Low	Primary (MRMP subset)
Oishi T 2019	2019	Japan (85 institutions)	2011–2016	Cross-sectional	1,256	873 (69.5%)	Pediatric	PPLO broth microdilution (Waites method; QC strain FH)	Low	Primary (MRMP subset)
Guo D 2019	2019	Beijing, China (11 hospitals)	2014	Cross-sectional	65	57 (87.7%)	Pediatric	Broth microdilution (breakpoints author-adapted from CLSI M100-S20)	Low	Primary (MRMP subset)
Wang Y 2016	2016	Hangzhou, China	2014 (school outbreak)	Outbreak investigation	18	18 (100%)	Pediatric	Broth dilution (CLSI M43-P)	Moderate	Primary
Wang N 2022	2022	Shanghai, China	2017–2019	Cross-sectional	182	72 (39.6%)	Pediatric (ages NR)	Broth microdilution (CLSI M43-A)	Low	Primary
Oishi T 2022	2022	Japan (85 institutions)	2017–2020	Cross-sectional	122 (MRMP 46)	46 (37.7%)	Pediatric	PPLO broth microdilution (non-CLSI)	Low	Primary (MRMP subset)
Yan C 2025	2025	10 provinces, China	Oct-Dec 2023	Cross-sectional	190	190 (100%)	Pediatric MPP	PPLO broth microdilution read per CLSI M43-A	Low	Primary
Yan C 2026	2026	Beijing, China	2017–2025	Cross-sectional	197	197 (100%)	Pediatric	Broth microdilution (CLSI M43-A; PPLO broth; QC strain M129)	Low	Primary
Wu Q 2026	2026	Shanghai, China	2023–2024	Cross-sectional	148 (146 AST-tested)	148 (100%)	Pediatric	Broth microdilution (CLSI M43-A; QC strain M129)	Low	Primary
Fang M 2026	2026	Jinan, China	2021–2024	Cross-sectional	227	227 (100%)	Pediatric	Broth microdilution (CLSI M43-A)	Low	Primary
Liu Y 2010	2010	Shanghai, China (single center)	March 2008-July 2009	Cross-sectional	100	90 (90.0%)	Pediatric (bronchial aspirations)	SP4 broth microdilution (Waites 2001 method; QC strain FH [ATCC 15531]; triplicates)	Moderate	Primary (MRMP subset)
Jiang Y 2024	2024	Multi-region (7 regions), China	2013–2019	Cross-sectional	4,145 specimens; 45 isolates MIC-tested	NR (MIC subset not age-stratified)	3,916 children + 229 adults	Broth microdilution (CLSI M43-A)	Low	Sensitivity-only
He WQ 2025	2025	Zhejiang, China	2022–2023	Cross-sectional	331 serology-diagnosed cases (no cultured isolates)	Not genotyped	Children ≤ 14 yr	"Serial dilution" (non-standardized)	High	Descriptive
Zhang D 2026	2026	Zibo, Shandong, China	2022–2023	Cross-sectional	49	NR	≤ 18 yr	Commercial kit (Autobio)	Moderate	Descriptive
Chen LJ 2025	2025	Chengde, Hebei, China	2021–2023	Cross-sectional	NR	NR	≤ 18 yr	Unspecified	High	Descriptive

Study	Year	Location	Period	Design	N	MRMP n (%)	Population	Susceptibility method	Risk of bias	Analysis group
Liu XJ 2014	2014	Beijing, China	2003–2007	Cross-sectional	76 (50 second-line-tested)	46 of 50 tested (92%)	Pediatric	Broth dilution (NCCLS 2006, non-M43-A)	Moderate	Descriptive
Shi RH 2026	2026	Zhengzhou, China	NR	Cross-sectional	72 (23 AST-tested)	72 (100%; all A2063G)	Hospitalized children	Dilution AST in a 23-isolate subset	Moderate	Descriptive
Wuzhou 2026	2026	Wuzhou, Guangxi, China	2023–2025	Cross-sectional	2,753 (1,759 MP+)	NR	Children 0–14 yr	Commercial culture + microdilution kit	Moderate	Descriptive
Morozumi M 2008	2008	Japan (multi-center)	2002–2006	Cross-sectional	380	NR (MIC summaries only)	Pediatric CAP	PPLO broth microdilution	Low	Descriptive (MIC summaries only)
Ozaki T 2007	2007	Japan	NR (pre-MRMP era)	Cross-sectional	14	NR	Pediatric	Broth microdilution	Moderate	Descriptive (MIC50/90 only)

Table 2
Study-level second-line resistance results (resistant/tested isolates, all cultured isolates by each study's own criteria).

Study	TET	DOXY	MINO	LEVO	MOXI	CIPRO	TOSU	SITA
Morozumi M 2005	NT	NT	0/12	0/12	NT	NT	NT	0/12
Oishi T 2019	0/1,256	NT	0/1,256	0/1,256	NT	NT	0/1,256	NT
Oishi T 2022	NT	NT	0/122	NT	NT	NT	NT	NT
Guo D 2019	0/65	NT	0/65	0/65	NT	NT	NT	NT
Wang Y 2016	MIC only*	NT	MIC only*	MIC only*	NT	MIC only*	NT	NT
Wang N 2022	0/182	0/182	NT	0/182	0/182	NT	NT	NT
Yan C 2025	0/190	0/190	NT	NT	0/190	NT	NT	NT
Yan C 2026	0/197	NT	NT	0/197	0/197	NT	NT	NT
Wu Q 2026	0/146	0/146	NT	0/146	0/146	NT	NT	NT
Fang M 2026	0/227	NT	NT	0/227	NT	NT	NT	NT
Liu Y 2010	0/90	0/90	0/90	0/90	0/90	0/90	NT	NT

*NT, not tested. Denominator is the number of cultured pediatric isolates tested against each agent by each study's own susceptibility criteria; all events = 0. *Wang Y 2016 (18 isolates) reported minimum inhibitory concentrations for tetracycline, minocycline, levofloxacin, and ciprofloxacin without a susceptible/resistant classification and was not pooled for any agent; its levofloxacin MICs ranged 0.125-4 ug/mL. Morozumi M 2005 provides isolate-level second-line data for 12 macrolide-resistant isolates. TET, tetracycline; DOXY, doxycycline; MINO, minocycline; LEVO, levofloxacin; MOXI, moxifloxacin; CIPRO, ciprofloxacin; TOSU, tosufloxacin; SITA, sitafloxacin.*

3.2 Primary Analysis: Drug-Specific Zero-Event Results

Drug-specific study-level and pooled results are summarized in Tables 3 and 4 and Figs. 2–4; full study-level and subgroup datasets are provided in Supplementary Tables 4 and 5. No resistant isolate was observed in any primary-analysis study for any drug. For tetracycline, 8 studies (Oishi T 2019, 0/1,256, Fang M 2026, 0/227, Yan C 2026, 0/197, Yan C 2025, 0/190, Wang N 2022, 0/182, Wu Q 2026, 0/146, Liu Y 2010, 0/90, Guo D 2019, 0/65; n = 2,353) reported no resistant isolate by each study's own susceptibility criteria, giving a pooled rate of 0.00% (exact upper 95% bound 0.16%; Fig. 2). In Fang M 2026, all 227 isolates had tetracycline MICs of ≤ 0.125-0.5 µg/mL, within the susceptible range, with no resistant isolate. For doxycycline, 4 studies (Wang N 2022, 0/182, Yan C 2025, 0/190, Wu Q 2026, 0/146, Liu Y 2010, 0/90; n = 608) reported no resistant isolate by each study's own susceptibility criteria, giving a pooled rate of 0.00% (exact upper 95% bound 0.60%). For levofloxacin, 8 studies (Morozumi M 2005, 0/12, Oishi T 2019, 0/1,256, Guo D 2019, 0/65, Wang N 2022, 0/182, Liu Y 2010, 0/90, Yan C 2026, 0/197, Wu Q 2026, 0/146, Fang M 2026, 0/227; n = 2,175) reported no resistant isolate by each study's own susceptibility criteria, giving a pooled rate of 0.00% (exact upper 95% bound 0.17%). Yan C 2025 did not test levofloxacin; the levofloxacin entry previously attributed to that study was incorrect. Wang Y 2016, a school-outbreak collection, reported levofloxacin MICs ranging 0.125-4 µg/mL (two of 18 isolates at 4 µg/mL) without an accompanying susceptible/resistant classification or *gyrA*/*parC* sequencing; because the study's own reference-susceptible strain M129 read 2 µg/mL and no quinolone-resistance breakpoint is established for *M. pneumoniae*, these isolates cannot be classified as resistant or susceptible and the study was reported descriptively rather than pooled. For moxifloxacin, 5 studies (Wang N 2022, 0/182, Yan C 2025, 0/190, Yan C 2026, 0/197, Wu Q 2026, 0/146, Liu Y 2010, 0/90; n = 805) reported no resistant isolate by each study's own susceptibility criteria, giving a pooled rate of 0.00% (exact upper 95% bound 0.46%). For minocycline, 5 studies (Morozumi M 2005, 0/12, Oishi T 2019, 0/1,256, Guo D 2019, 0/65, Oishi T 2022, 0/122, Liu Y 2010, 0/90; n = 1,545) reported no resistant isolate by each study's own susceptibility criteria, giving a pooled rate of 0.00% (exact upper 95% bound 0.24%). Minocycline results from a further three studies were not classifiable because no breakpoint exists: Wang N 2022 tested 182 isolates (the full tested set, including the 5

macrolide-susceptible isolates; MIC range 0.03-4 mg/L; five isolates [2.7%] with MIC = 4 mg/L), Wu Q 2026 tested 146 isolates (all MICs ≤ 0.25 mg/L), and Wang Y 2016 tested 18 isolates (MIC range 0.03125-0.5 μg/mL); these are reported as MIC distributions only and were not pooled. For tosufloxacin, two Japanese studies reported zero resistant MRMP isolates (Oishi T 2019, 0/873; Oishi T 2022, 0/46; n = 919; pooled 0.00%, upper bound 0.40%). Sitafloxacin was tested only by Morozumi M 2005 (0/12; descriptive). For ciprofloxacin, the newly included Liu Y 2010 is the only primary-analysis study, reporting zero resistant isolates among 90 MRMP isolates (0/90; exact upper 95% bound 4.02%); this single-study result is reported descriptively and was not pooled. Because all primary analyses contained only zero-event studies, between-study heterogeneity (I^2) was not estimable; under such conditions an I^2 of 0% is uninformative rather than evidence of homogeneity.

Table 3
Pooled second-line resistance: primary analysis (all cultured isolates), secondary analysis (macrolide-resistant isolates), and macrolide-susceptible comparison.

Drug	Analysis	k	n	Events	%	Exact upper 95%
Tetracycline	Primary (all isolates)	8	2,353	0	0.00	0.16
	Secondary (MRMP only)	8	1,852	0	0.00	0.20
	Macrolide-susceptibleb	8	501	0	0.00	0.73
Levofloxacin	Primary (all isolates)	8	2,175	0	0.00	0.17
	Secondary (MRMP only)	8	1,674	0	0.00	0.22
	Macrolide-susceptibleb	8	501	0	0.00	0.73
Minocycline	Primary (all isolates)	5	1,545	0	0.00	0.24
	Secondary (MRMP only)	5	1,078	0	0.00	0.34
	Macrolide-susceptibleb	5	467	0	0.00	0.79
Moxifloxacin	Primary (all isolates)	5	805	0	0.00	0.46
	Secondary (MRMP only)	5	695	0	0.00	0.53
	Macrolide-susceptibleb	5	110	0	0.00	3.30
Doxycycline	Primary (all isolates)	4	608	0	0.00	0.60
	Secondary (MRMP only)	4	498	0	0.00	0.74
	Macrolide-susceptibleb	4	110	0	0.00	3.30

Primary analysis uses all cultured pediatric M. pneumoniae isolates tested against each agent, classified by each study's own criteria. Secondary analysis restricts the denominator to macrolide-resistant (MRMP) isolates. bThe macrolide-susceptible row covers isolates reported as macrolide-susceptible within studies that classified all isolates (Oishi T 2019, which tabulated tetracycline and fluoroquinolone MICs separately for its macrolide-susceptible and macrolide-resistant groups; Oishi T 2022; Wang N 2022; Guo D 2019). Where a study reported second-line results at the cohort level (no resistant isolate detected across the full collection) rather than itemizing every macrolide-susceptible isolate, the macrolide-susceptible count is a cohort-level inference and its exact upper bound should be read as approximate. Wang Y 2016 is excluded from pooling (minimum inhibitory concentrations reported without classification). Zero resistant isolates were observed in every stratum.

Table 4
Molecular markers, breakpoints, and study-design notes for descriptive-analysis studies.

Study	23S rRNA	gyrA	parC	Breakpoints used	Key findings / design note
Morozumi M 2005	Sequencing (A2063G/A2064G)	NR	NR	PPLO broth microdilution; QC strain M129; contemporary interpretive criteria	Zero resistance to minocycline, levofloxacin, and sitafloxacin among 12 MRMP isolates
Oishi T 2019	Sequencing	NR	NR	PPLO broth microdilution (Waites method); QC strain FH	Zero resistance to TET, MINO, LEVO, and TOSU among 873 MRMP isolates ("strains resistant to these agents were not detected")
Guo D 2019	Sequencing	NR	NR	Author-adapted from CLSI M100-S20 (flagged in quality assessment)	Zero resistance to TET, MINO, and LEVO among 57 MRMP isolates
Wang Y 2016	PCR + sequencing (A2063G)	NR	NR	CLSI M43-P broth dilution; levofloxacin susceptible-only cutoff ≤ 1 μg/mL	Reported minimum inhibitory concentrations only, without a susceptible/resistant classification; not pooled for any agent. TET MIC 0.0625-0.5, MINO MIC 0.03125-0.5, LEVO MIC 0.125-4 (2/18 isolates at 4 ug/mL, above the susceptible-only cutoff of 1 ug/mL; reference strain M129 read 2 ug/mL), CIPRO tested. No gyrA/parC sequencing.
Wang N 2022	Sequencing	NR	NR	ERY S ≤ 0.5/R ≥ 1; TET S ≤ 2; DOXY S ≤ 2; LEVO S ≤ 1; MOXI S ≤ 0.25 mg/L; MINO: no CLSI breakpoint	Zero resistance to all tested second-line agents (182 cultured isolates tested, of which 72 were macrolide-resistant); minocycline not classifiable (182 isolates tested; MIC range 0.03-4 mg/L)
Yan C 2025	Sequencing (A2063G, 100%)	NR	NR	Per CLSI M43-A	MRMP 100%; zero resistance to TET, DOXY, and MOXI
Yan C 2026	PCR + sequencing (A2063G 100%)	NR	NR	TET S ≤ 2; LEVO S ≤ 1; MOXI S ≤ 0.5 μg/mL (CLSI M43-A)	100% MRMP; zero resistance to TET, LEVO, and MOXI among 197 isolates (MIC90 0.5/1/≤0.125 μg/mL)
Wu Q 2026	Whole-genome sequencing (A2063G 100%)	NR	NR	TET S ≤ 2; DOXY S ≤ 2; LEVO S ≤ 1; MOXI S ≤ 0.5 mg/L (CLSI M43-A); MINO: no CLSI breakpoint	100% MRMP; zero resistance to TET, DOXY, LEVO, and MOXI among 146 tested isolates; minocycline MICs all ≤ 0.25 mg/L (not classifiable, MIC distribution only)
Fang M 2026	PCR (A2063G 100%)	NR	NR	TET S ≤ 2; LEVO S ≤ 1 (CLSI M43-A)	Zero resistance to TET (MIC ≤ 0.125-0.5 μg/mL) and LEVO (MIC ≤ 0.125-1 μg/mL) among 227 isolates
Liu Y 2010	Sequencing (A2063G 88/90 [98%], A2064G 1/90, A2063T 1/90)	NR	NR	SP4 broth microdilution (Waites 2001); FH (ATCC 15531) reference control; triplicates; CLSI provides no breakpoints for second-line agents, susceptible-only criteria	MRMP 90/100 (90%); all isolates susceptible to tetracycline, doxycycline, minocycline, ciprofloxacin, levofloxacin, and moxifloxacin; zero resistance among the 90 MRMP isolates (MIC ranges [μg/mL]: TET 0.015–0.25, DOXY ≤ 0.007–0.125, MINO ≤ 0.007–0.125, CIPRO 0.125-1, LEVO 0.125-0.5, MOXI 0.03–0.125)
Oishi T 2022	NR (not performed)	NR	NR	MRMP defined by bimodal CAM/AZM MIC distribution; explicit breakpoints NR	Zero minocycline and tosufloxacin resistance among 46 MRMP isolates
Jiang Y 2024	Sequencing (A2063G, A2064G)	NR	NR	No CLSI TET/FQ resistance breakpoints; MICs ≤ 0.5 (TET) and ≤ 1 μg/mL (LEVO)	0/45 MIC-tested isolates resistant to TET and LEVO; sensitivity analysis only
He WQ 2025	NR (not performed)	Sequencing	Sequencing	No CLSI standard, breakpoints, or QC strain	Serological diagnosis (passive agglutination), no cultured isolates; tabulated fluoroquinolone/doxycycline percentages are not isolate-based resistance rates and were excluded from synthesis
Zhang D 2026	NR	NR	NR	Commercial kit criteria	Limited gene detection data
Chen LJ 2025	NR	NR	NR	NR	IgM serology, no isolates; disc diffusion (no M. pneumoniae breakpoints); qualitative only
Liu XJ 2014	Sequencing (same 50-isolate set as Xin D 2009)	NR	NR	NCCLS 2006 (non-M43-A)	TET 0/50 and CIPRO 0/50; 5 isolates with ciprofloxacin MIC = 2 μg/mL of ambiguous interpretation
Shi RH 2026	PCR (A2063G 100%)	NR	NR	Study-level criteria; AST subset only	Zero resistance to fluoroquinolones and tetracyclines (class-level) among 23 tested isolates
Wuzhou 2026	NR	NR	NR	Commercial kit per manufacturer	Fluoroquinolone resistance < 0.5% among 1,759 culture isolates (LEVO 0.45%, GAT 0.23%, SPA 0.11%)
Morozumi M 2008	Sequencing	NR	NR	PPLO broth microdilution	380 pediatric isolates; MIC summaries only, no isolate-level resistance counts; partial overlap with Morozumi M 2005

Study	23S rRNA	gyrA	parC	Breakpoints used	Key findings / design note
Ozaki T 2007	NR (pre-MRMP era)	NR	NR	Broth microdilution	14 pediatric isolates; MIC50/90 summaries only

3.3 Sensitivity Analysis

Adding Jiang Y 2024 (tetracycline 0/45 and levofloxacin 0/45 MIC-tested isolates) to the corresponding primary analyses did not change any conclusion: the pooled resistance rate remained 0.00% for tetracycline (k = 9, n = 2,398; exact upper bound 0.15%) and for levofloxacin (k = 9, n = 2,220; exact upper bound 0.17%). The age and MRMP status of these 45 isolates could not be confirmed; this analysis tests only the effect of denominator expansion on the upper bound. Because the Beijing subset of Yan C 2025 (24 of its 190 isolates) may overlap with the 2017–2025 Beijing collection of Yan C 2026, we repeated the affected analyses after excluding Yan C 2025. All estimates remained 0.00%: tetracycline (k = 7, n = 2,163; upper bound 0.17%), moxifloxacin (k = 4, n = 615; upper bound 0.60%), and doxycycline (k = 3, n = 418; upper bound 0.88%). Because every contributing study observed zero events, any such overlap (≤ 24 isolates) cannot introduce resistant isolates into the pooled denominators, and the conclusions were unchanged. Finally, because Akaike H 2012 was excluded as an overlapping cohort with Oishi T 2019 on the basis of a potential eight-month overlap (January-August 2011) within the same surveillance network, we repeated the analyses it could have contributed to while disregarding the overlap. Adding its all-zero results (124 MRMP isolates tested against tetracycline and minocycline) again left every estimate at 0.00%: tetracycline n = 2,099 (upper bound 0.18%) and minocycline n = 1,202 (upper bound 0.31%). Conclusions were unchanged in all sensitivity scenarios. As a worst-case bound on the one study that reported levofloxacin MICs without a susceptible/resistant classification, we provisionally reclassified the two Wang Y 2016 isolates with levofloxacin MIC = 4 ug/mL as resistant and returned all 18 of its isolates to the levofloxacin denominator; the pooled levofloxacin rate became 2/2,193 (0.09%) with an exact upper 95% bound of 0.33%, still below 0.5% and leaving the conclusion unchanged.

3.4 Descriptive Analysis (Non-Standardized, Non-Isolate-Based, or Summary-Level Studies)

He WQ 2025 (n = 331 children ≤ 14 years) diagnosed *M. pneumoniae* infection serologically (passive particle agglutination, titer ≥ 1:160) and did not culture or genotype any isolate. The report nevertheless tabulated drug-specific "resistance" of 3.02% for doxycycline, 4.83% for levofloxacin, 3.32% for moxifloxacin, and 7.25% for ciprofloxacin. Because susceptibility and resistance are properties of a viable isolate measured against defined breakpoints, and this study had no isolates, no reference method, and no quality-control strain, these percentages cannot be interpreted as *M. pneumoniae* resistance rates; they are not a resistance signal and were not pooled, plotted, or compared with isolate-based data. We retain this study only as a documented example of a methodological problem recurrent in the pediatric *M. pneumoniae* literature, resistance figures reported from designs that cannot measure resistance, and summarize its design alongside the other descriptive-analysis studies in Table 4 (study-design quality). Five other descriptive-analysis studies reported low or no second-line resistance with important methodological caveats. Liu XJ 2014 (Beijing, 2003–2007; the same 50-isolate set as Xin D 2009 [40]) was assigned to the descriptive analysis for two reasons: testing followed NCCLS 2006 criteria rather than CLSI M43-A, and the second-line-tested subset (50 isolates, 92% MRMP) was not stratified by MRMP status, so an MRMP-stratified denominator could not be extracted; it found 0/50 resistant to tetracycline and ciprofloxacin, with five isolates at a ciprofloxacin MIC of 2 μg/mL of ambiguous interpretation. Shi RH 2026 (Zhengzhou; SP4 broth microdilution with the M129 (ATCC 29342) quality-control strain and CLSI M43-A interpretation, performed on a 23-isolate subset of 72 characterized isolates, all carrying A2063G) reported 0/23 resistant to fluoroquinolones and tetracyclines at the class level. Although its method was standardized (SP4 broth microdilution, M129 quality-control strain, CLSI M43-A), susceptibility was reported for only 23 of the 72 characterized isolates; this small subset yields a wide and unstable exact confidence interval (upper bound 14.8% at the class level), so the study was placed in the descriptive analysis rather than the primary analysis. The method itself was not the limiting factor. Morozumi M 2008 (380 pediatric isolates, Japan, 2002–2006, partially overlapping the Morozumi M 2005 collection) and Ozaki T 2007 (14 pediatric isolates, pre-MRMP-era) reported MIC summaries (MIC50/MIC90) without isolate-level resistance counts, precluding pooling; Morozumi M 2008 is retained as descriptive evidence because its partial overlap with Morozumi M 2005 cannot affect any pooled denominator, summary-level MIC data never enter pooling. Wuzhou 2026 (Wuzhou, Guangxi, 2023–2025; 2,753 children screened, 1,759 culture-positive isolates tested by a commercial rapid culture-and-susceptibility kit) found fluoroquinolone resistance below 0.5% (levofloxacin 8/1,759, 0.45%; gatifloxacin 4/1,759, 0.23%; sparfloxacin 2/1,759, 0.11%); because the kit is not a CLSI reference method, these isolate-level results are reported as descriptive evidence concordant with the primary analysis. Zhang D 2026 (Zibo; 49 culture-positive isolates tested with a commercial culture-based susceptibility kit) reported 0% resistance to all six second-line agents tested, including minocycline, doxycycline, ciprofloxacin, moxifloxacin, levofloxacin, and gatifloxacin (0/49 each); because the kit is not a CLSI reference method, this isolate-level result is reported as descriptive evidence. Chen LJ 2025 diagnosed infection by IgM serology without culturing isolates and performed disc diffusion, a method for which no *M. pneumoniae* breakpoints exist, so only a qualitative statement (no doxycycline resistance) could be extracted; it is retained as descriptive evidence only. These studies are summarized in Table 4.

3.5 Publication Bias

Formal assessment of publication bias was not performed because every drug-specific primary analysis included at most nine studies, all with zero events; funnel-plot-based assessments are underpowered and uninformative in this setting [10, 11]. The funnel plot presented in the previous version of this review was removed.

In a structured evidence-quality summary (Supplementary Table 4), the body of evidence was strong for sample size and statistical precision (more than 2,000 isolates per class for tetracyclines and fluoroquinolones, with exact upper bounds at or below 0.17%) and for consistency and representativeness (a uniform zero finding across China and Japan and across 2002 to 2026), of moderate methodological quality by JBI appraisal (standardized dilution with reference strains), and limited by non-uniform interpretive breakpoints, which a sensitivity analysis restricted to CLSI M43-A studies showed did not alter the zero-resistance finding.

Discussion

In this updated synthesis, no resistance to any second-line agent was observed among cultured pediatric isolates tested with standardized dilution methods: across 2,353 tetracycline-tested, 608 doxycycline-tested, 2,175 levofloxacin-tested, and 805 moxifloxacin-tested isolates, not a single resistant isolate was detected; ciprofloxacin likewise showed zero resistance (0/90) in the only standardized pediatric dataset (Liu Y 2010). This absence of detected resistance spans 23 years (2002–2025) and two countries with historically high MRMP prevalence: the Japanese collections begin with Morozumi M 2005 (2002–2004) and the Chinese collections extend through Yan C 2026 (2017–2025), covering successive epidemic periods, multiple surveillance networks, and both national multicenter cohorts and single-outbreak collections. Our quantitative finding is consistent with two decades of narrative and surveillance evidence in both children and adults. Comprehensive reviews of *M. pneumoniae* antimicrobial resistance have repeatedly stated that, in contrast to the global rise of macrolide resistance, tetracycline and fluoroquinolone resistance has not been reported in clinical *M. pneumoniae* isolates [45]. Population-level surveillance supports this: in a Chinese multicenter study of adults with community-acquired pneumonia, no isolate was resistant to fluoroquinolones or tetracyclines despite a high macrolide-resistance rate, leading the authors to conclude that macrolide-resistant infection can be treated effectively with a fluoroquinolone [44]; longitudinal monitoring in Beijing from 2008 to 2012 found all isolates susceptible to fluoroquinolones and tetracyclines even as macrolide resistance exceeded 90% [43]; and surveillance in Ontario, Canada, identified no fluoroquinolone- or tetracycline-resistant specimens and no *gyrA* or 16S rRNA resistance mutations [46]. Against this literature, the present review contributes a pooled, isolate-level quantification: a combined upper 95% confidence bound of 0.16–0.17% for tetracycline and levofloxacin across more than 2,000 cultured isolates, converting a widely repeated qualitative impression into an explicit bound on how rare second-line resistance can be in this population. The genetic independence of the underlying mechanisms ensures that macrolide pressure does not co-select second-line resistance: macrolide resistance is conferred by 23S rRNA domain V mutations, whereas fluoroquinolone resistance requires *gyrA* and *parC* quinolone-resistance-determining-region mutations and tetracycline resistance requires ribosomal protection or efflux determinants, none of which are selected by macrolide pressure. This independence prevents second-line agents from being dragged down alongside the macrolides, but it is not by itself the reason their resistance remains near zero; the low clinical prevalence is driven primarily by weak selection pressure, because fluoroquinolones are generally avoided in children and tetracyclines are restricted in younger ages, so the de novo mutations that laboratory selection can produce are rarely selected in treated children. The pediatric focus of this review reflects the epidemiology of *M. pneumoniae* rather than a limitation of scope. *M. pneumoniae* is a leading cause of community-acquired pneumonia in school-aged children and adolescents: in the U.S. EPIC study of radiographically confirmed pneumonia requiring hospitalization, it was detected far more often in children 5 years of age or older than in younger children (19% versus 3%), and among hospitalized school-aged children it is a pathogen that should be routinely considered [49, 50]. This age distribution, with peak incidence between roughly 5 and 15 years and relative sparing of infants, is a long-recognized feature of the organism's biology and transmission in school settings [51]. Because the burden of *M. pneumoniae* pneumonia, and therefore the clinical pressure to use second-line agents when macrolides fail, is concentrated in this age band, pediatric isolates are the population in which second-line resistance would first and most consequentially emerge. The absence of such resistance is not confined to children: surveillance of adult community-acquired pneumonia has likewise found no fluoroquinolone or tetracycline resistance despite high macrolide resistance, and comprehensive reviews across all ages report that resistance to these classes has not been documented in clinical *M. pneumoniae* isolates [43, 44, 45]. The concordance between pediatric and adult data strengthens the inference that retained second-line activity is a property of the organism and its resistance mechanisms rather than an artifact of the pediatric sampling frame. A mechanistic asymmetry explains why second-line resistance remains rare in *M. pneumoniae* while it is common in many other bacterial pathogens. In Enterobacterales, staphylococci, and pneumococci, tetracycline resistance is overwhelmingly acquired horizontally through mobile genetic elements that carry ribosomal-protection genes (tet(M), tet(O)) or efflux determinants on plasmids, transposons, and integrons, and fluoroquinolone resistance is reinforced by plasmid-borne qnr genes and efflux up-regulation in addition to target mutations. *M. pneumoniae* has a small (~ 0.8 Mb) genome, lacks plasmids and known conjugative or transposable elements, and propagates essentially clonally, so horizontal gene transfer is not a major route of resistance evolution in this species and it has little efficient means to import these resistance genes; second-line resistance in this organism would therefore have to arise de novo through *gyrA* and *parC* quinolone-resistance-determining-region mutations (for fluoroquinolones) or through acquisition of ribosomal-protection or efflux determinants (for tetracyclines). Such de novo events are further constrained by weak selective pressure, because fluoroquinolones are generally avoided in children and tetracyclines are restricted in younger children, so the drug exposure that enriches resistant subpopulations in other species is largely absent. The same framework explains the contrasting high macrolide-resistance rate: a single 23S rRNA domain V mutation (most often A2063G) confers full macrolide resistance in an organism with only one or two rRNA operons, and macrolides are the heavily used first-line agents, combining a low genetic barrier with strong selection. This asymmetry is consistent with the parallel emergence of clinical fluoroquinolone resistance in *Mycoplasma genitalium* [47] and of novel quinolone-resistance mutations in *Mycoplasma bovis* [48], which shows that the genus is not intrinsically incapable of second-line resistance; continued surveillance is therefore warranted should pediatric second-line use increase. The biological potential for second-line resistance, as distinct from its current clinical absence, is well documented at the strain level. *In vitro*, stepwise exposure of *M. pneumoniae* to fluoroquinolones readily selects resistant mutants carrying quinolone-resistance-determining-region substitutions in *gyrA*, *gyrB*, *parC*, and *parE*, with parallel increases in minimum inhibitory concentrations across the class [52]. Where resistance has been selected, the responsible changes cluster in a small, conserved target region, the quinolone-resistance-determining region of the topoisomerase genes, consistent with the limited and recurrent set of positions seen across Mycoplasma species; in *in vitro*-selected *M. pneumoniae* mutants the primary target varies with the agent, with ciprofloxacin and sparfloxacin selecting *parC* changes and levofloxacin and moxifloxacin selecting *gyrA* or *gyrB* changes, and *gyrB* mutations appearing comparatively prominent in this species [52, 53]. This concentration of resistance determinants in a short, well-defined region is what makes a molecular assay tractable. It should be noted, however, that because clinical fluoroquinolone resistance in *M. pneumoniae* is so rare, the species-specific spectrum and exact codon positions of resistance mutations have not been as fully catalogued as for organisms under heavier fluoroquinolone pressure, so a surveillance panel would need to sequence the whole quinolone-resistance-determining region rather than probe a single pre-specified codon. Characterizations of such laboratory-derived and reference strains have mapped the specific topoisomerase and ribosomal changes that confer tetracycline and fluoroquinolone resistance, confirming that the genetic routes to resistance exist and are accessible under selective pressure even though they are not conferred by the macrolide-resistance mutations that dominate current clinical isolates [53]. Experimental evolution of tetracycline resistance through ribosomal and efflux changes has likewise been demonstrated [41]. These strain-level findings,

which are independent of the geographic or clinical origin of the isolate, reconcile the uniform clinical zero with a realistic expectation: second-line resistance is not precluded by the organism's biology but has simply not yet been selected in treated children, most plausibly because fluoroquinolone and tetracycline exposure in this population remains limited. This is the mechanistic basis for ongoing phenotypic and molecular surveillance rather than for complacency. Whether this surveillance warrants a dedicated second-line resistance assay deserves explicit consideration. The practical obstacle to current monitoring is that phenotypic susceptibility testing of *M. pneumoniae* requires culture, which is slow, technically demanding, and available in few laboratories, so most clinical services never test second-line agents at all; this is the main reason isolate-level data remain sparse despite decades of macrolide-resistance surveillance. Because the resistance determinants are well defined and few, specifically the *gyrA* and *parC* quinolone-resistance-determining regions for fluoroquinolones and ribosomal-protection or efflux and 16S rRNA changes for tetracyclines, a culture-independent molecular assay, for example a multiplex PCR or targeted-sequencing panel applied directly to respiratory specimens, is technically feasible and would bypass the culture bottleneck. At present, however, the near-zero prevalence documented here means the pre-test probability of second-line resistance is very low, so a stand-alone diagnostic assay for routine clinical use would have limited positive predictive value and is not yet justified. A more proportionate approach is to add the second-line resistance markers to the existing macrolide-resistance (23S rRNA) molecular assays that are already used widely in East Asia, creating a combined panel for sentinel surveillance rather than universal testing. Such a panel would allow large-scale, culture-independent monitoring that could detect the earliest emergence of *gyrA* or *parC* mutations, converting the present reassuring but zero-event picture into an active early-warning system without the cost and low yield of a dedicated routine test. The main clinical implication is not that second-line resistance is absent, but that the available pediatric culture-based data have not yet shown widespread resistance to these agents. This distinction is important because *M. pneumoniae* culture and susceptibility testing are technically demanding, performed in relatively few centers, and often reported with small drug-specific denominators. The evidence base is therefore useful for surveillance and hypothesis generation, but it remains insufficient for strong comparative treatment recommendations.

The updated search substantially strengthened the evidence base, enlarging the qualitative synthesis from nine to twenty pediatric studies and the primary analysis from five to eleven. It also resolved the status of Liu Y 2010, initially left unclassified pending full-text access: the full text was obtained and verified during this revision, adding a standardized Shanghai pediatric collection (100 isolates, 90 MRMP) with six second-line drugs tested. It contributed the two largest datasets in the review, Oishi T 2019 (1,256 isolates, 873 MRMP, 85 institutions) and Fang M 2026 (227 isolates), together with the earliest collections (Morozumi M 2005, 2002–2004; Ozaki T 2007) and the first outbreak-based collection (Wang Y 2016), extending the tetracycline denominator from a few hundred to 2,353 isolates. The updated search also surfaced Huang C 2025, which we excluded but report as a cautionary example [35]: its commercial colorimetric kit, designed for Ureaplasma and *M. hominis* rather than *M. pneumoniae*, applied a 24–48 h incubation incompatible with this slow-growing organism, provided no breakpoints or MICs; its implausible results (38.6% tetracycline and 31.4% quinolone resistance, with triple 23S rRNA mutations reported in 100% of isolates and internal contradictions) are consistent with method invalidity and corroborate, rather than motivate, the exclusion. Zhang D 2026 likewise relied on a commercial kit and could therefore contribute only descriptive (qualitative) data [24]; together with the excluded Huang C 2025, it illustrates why non-validated kit results should not enter resistance syntheses, and why unusually high resistance rates generated by non-validated methods should trigger methodological scrutiny rather than alarm. A commercial-kit surveillance from Wuzhou (2026), which tested 1,759 culture-positive pediatric isolates, is consistent with this pattern: when the assay is a rapid culture-and-susceptibility kit applied to cultured isolates rather than an assay designed for other organisms, fluoroquinolone resistance remained below 0.5% (levofloxacin 0.45%, gatifloxacin 0.23%, sparfloxacin 0.11%), concordant with the standardized primary analysis and reported here as descriptive evidence. This distinction, commercial kits designed or validated for *M. pneumoniae* contribute descriptive (qualitative) evidence, whereas assays designed for other organisms with incompatible incubation conditions are methodologically invalid, underlies the different handling of these studies.

The observed concordance is biologically coherent with mechanistic literature. In a recent in-vitro induction study, tetracycline resistance could be selected only under sustained experimental pressure and has never been reported in clinical *M. pneumoniae* isolates [41]; killing-kinetics experiments confirm rapid bactericidal activity of minocycline, doxycycline, and tosufloxacin against MRMP [37]; and pharmacodynamic modeling of nemonoxacin and comparators supports preserved fluoroquinolone potency against contemporary clinical isolates [36]. The biological plausibility of preserved second-line activity is strong. Macrolide resistance in *M. pneumoniae* is usually associated with 23S rRNA mutations, whereas reduced susceptibility to tetracyclines and fluoroquinolones involves different targets [1, 6]. This lack of direct cross-resistance supports the continued role of tetracyclines and fluoroquinolones as potential alternatives after macrolide failure. However, preserved *in vitro* susceptibility should not be equated with clinical superiority. Defervescence, radiographic progression, host inflammatory response, timing of therapy, and severity at presentation all influence outcomes in pediatric pneumonia, and these factors were not consistently available in the included susceptibility reports.

Two signals nonetheless warrant continued surveillance. Wang Y 2016 reported two of 18 outbreak isolates with levofloxacin MIC = 4 μg/mL, above the susceptible-only cutoff, the first such non-susceptibility signal in a pediatric MRMP collection, albeit within a small, clonal school-outbreak sample and without an established resistance breakpoint. This isolated observation is the only elevated second-line MIC in the entire body of evidence and warrants molecular investigation (*gyrA* and *parC* sequencing) to distinguish emerging quinolone resistance from laboratory-condition variation; it should not be dismissed, but neither can it be counted as confirmed resistance. By contrast, the percentages tabulated by He WQ 2025 (ciprofloxacin 7.25%, with lower figures for levofloxacin, moxifloxacin, and doxycycline) cannot be read as a resistance signal at all: that study diagnosed infection serologically and cultured no isolate, so there was no organism on which susceptibility could be measured. Resistance is a property of a viable isolate tested against defined breakpoints; a serologic case series has no such denominator, and we therefore excluded these figures from every quantitative comparison and from Fig. 3. The genuine signal that upper-bound surveillance is designed to detect is the isolate-based one from Wang Y 2016.

A dedicated caution concerns the gap between *in vitro* susceptibility and clinical efficacy. *In vitro* activity is necessary but not sufficient for treatment success: drug exposure at the site of infection, host inflammatory response, timing of therapy, and pediatric safety profiles all influence outcomes, and none of the included studies reported clinical endpoints. Macrolides therefore remain the first-line treatment for pediatric *M. pneumoniae* pneumonia; tetracyclines and fluoroquinolones should be reserved for selected children with documented macrolide failure or contraindications, within age-appropriate safety constraints and antimicrobial-stewardship principles [6, 8].

Interpretation should also account for pediatric prescribing constraints. Tetracyclines may be attractive microbiologically because no resistant isolate was observed across 2,353 tested isolates, but their use in children is shaped by age-related safety considerations and local policy. Fluoroquinolones may be options in selected children when macrolides fail or cannot be used, yet concerns about adverse effects and antimicrobial stewardship mean that they should not be treated as routine first substitutes solely on the basis of *in vitro* susceptibility [6, 8]. In practical terms, these constraints translate into an age-dependent choice once a macrolide-resistant infection is suspected on clinical grounds: tetracyclines (minocycline, doxycycline) are generally reserved for older children, commonly from eight to twelve years of age depending on local policy, so for school-aged children who fall below those thresholds a fluoroquinolone is often the only remaining option, whereas for the youngest children no well-established second-line agent is available and management still depends on optimizing macrolide therapy and supportive care. This choice should be made only when inadequate response to an appropriate-dose, full-course macrolide marks probable resistance, since organism-level susceptibility data are rarely available at the point of care, and it should be paired with antimicrobial-stewardship review rather than a blanket switch. The present findings therefore support susceptibility surveillance rather than automatic escalation to a specific second-line agent.

The figures tabulated by He WQ 2025 (ciprofloxacin 7.25%, 24/331; levofloxacin 4.83%; moxifloxacin 3.32%; doxycycline 3.02%) deserve explicit comment precisely because they should not be mistaken for a resistance signal. That study diagnosed *M. pneumoniae* serologically (passive agglutination, antibody titer ≥ 1:160) and cultured no organism, so there was no isolate on which susceptibility could be determined; MRMP status was never genotyped; and the "serial dilution" procedure had no CLSI breakpoints, no reference method, and no quality-control strain. Antibody titers measure the host immune response, not the susceptibility of the organism, and the two are not connected by any validated testing path, so a percentage derived this way has no interpretable numerator-denominator relationship as a resistance rate. The appropriate comparator makes the point: the only standardized pediatric ciprofloxacin dataset, Liu Y 2010, found 0/90 resistant MRMP isolates by SP4 broth microdilution with a reference strain. We therefore treat He WQ 2025 not as weak evidence of resistance but as an example of a recurring design problem, resistance percentages reported from studies that cannot measure resistance, and we excluded its figures from all pooling, plotting, and comparison and molecular confirmation of both MRMP status and quinolone-resistance determinants.

Culturing versus serology: a measurement category distinction. A broader methodological distinction underlies these descriptive-analysis observations and merits explicit statement, because it, rather than any judgment of study quality, is why the serology-based figures were not pooled with the culture-based estimates. Antimicrobial susceptibility testing is defined on a viable, cultured isolate: a minimum inhibitory concentration is a property of an organism grown in the presence of drug and interpreted against a breakpoint, and the reference method for *Mycoplasma pneumoniae* is broth microdilution under CLSI M43-A. A serological diagnosis establishes only that a child mounted an antibody response to *M. pneumoniae*; the antibody titer reflects past or mixed infection and is not connected to the susceptibility phenotype of any isolate by a validated testing pathway. He WQ 2025 and Chen LJ 2025 diagnosed infection serologically and cultured no isolate, so the percentages they tabulate do not estimate the same quantity as an isolate-based resistance rate, they answer a different question and cannot enter the same quantitative synthesis. Chen LJ 2025 compounds this by applying disc diffusion, for which neither CLSI nor ISO has established *M. pneumoniae* breakpoints; the method is not applicable to this organism. We therefore separated culture-confirmed, dilution-based studies (the primary and sensitivity analyses) from serology-based or non-standardized studies (the descriptive analysis) as a prespecified eligibility distinction, and we report both strata transparently rather than discarding the serological data. Read this way, the contrast is itself informative: every non-zero second-line signal in this body of literature arises from a study that could not, by its design, measure isolate-level resistance, whereas all sixteen culture-and-dilution studies converge on zero or near-zero resistance.

The substantial heterogeneity reported in the levofloxacin analysis of the previous version was an artifact: re-extraction against the original publications showed that it was driven by the erroneous inclusion of He WQ 2025 in the primary analysis. With the corrected dataset, all primary analyses contain only zero-event studies, and between-study heterogeneity is not estimable; the absence of heterogeneity statistics should therefore not be misread as proven consistency across settings.

Zero-event studies are another important methodological issue. In antimicrobial-resistance surveillance, zero resistant isolates can be clinically reassuring, but a zero-event finding is not proof that the true resistance prevalence is zero. The upper bound of uncertainty is determined by the denominator: with 2,353 tetracycline-tested isolates and zero events, a true resistance rate of up to 0.16% remains statistically compatible with the data; for levofloxacin (n = 2,175) the corresponding bound is 0.17%, for doxycycline (n = 608) it is 0.60%, and for the 1,545 isolates tested against minocycline it is 0.24%. Low-frequency resistance of clinical relevance could therefore remain undetected, which is precisely why ongoing standardized surveillance matters: only larger, repeatedly sampled denominators can shrink these upper bounds and detect emergence early. Continuity corrections make rare-event meta-analysis possible, but they can also influence pooled estimates when many studies have no events. For this reason, the pooled estimates in this review should be read together with the study counts, denominators, and the exact upper bounds reported for each drug.

The geographic scope of the evidence remains narrow. The primary analysis rests on eight studies from China and three from Japan, and all twenty included studies were conducted in East Asia, with surveillance spanning 2002–2025; the title of this review has been amended accordingly. MRMP prevalence, second-line antibiotic exposure, and laboratory capacity differ across regions, so these zero-event findings should not be extrapolated to Europe, the Americas, or other settings where pediatric MRMP is also reported.

The descriptive-analysis studies using non-standardized methods were not pooled with the primary studies. Their separation is important because commercial or inadequately described methods may apply different interpretive criteria, have different quality-control procedures, and produce results that are not directly comparable with CLSI-based testing [39]. In rare resistance settings, even small differences in breakpoints, inoculum, culture conditions, or interpretation can change whether an isolate is classified as susceptible, intermediate, or resistant. Future studies should therefore report the testing standard, MIC distributions, drug-specific denominators, resistant numerators, and quality-control procedures in enough detail to allow independent synthesis.

This review has several limitations. First, each drug-specific primary analysis included at most nine studies, all with zero events, so precision is bounded by the available denominators and no heterogeneity could be estimated. Because conventional between-study variance measures (I-squared, Cochran Q) are undefined when every study reports zero events, we combined the per-study denominators and computed a single exact Clopper-Pearson upper bound; this pooled-denominator approach assumes that the contributing studies estimate a common underlying resistance rate. A further caveat concerns the gap between *in vitro* susceptibility and clinical efficacy: a susceptible minimum inhibitory concentration does not by itself guarantee a cure, because tissue penetration, pharmacokinetic and pharmacodynamic factors, and host response also determine outcome, and sporadic treatment failures with second-line agents have been reported in children who nonetheless carried phenotypically susceptible isolates. The uniform *in vitro* susceptibility documented here therefore supports these agents as microbiologically reliable options when macrolides fail, but should be interpreted as evidence about the organism's resistance phenotype rather than a direct measure of clinical response. That homogeneity assumption cannot be tested statistically here, but the studies span different countries (China and Japan), collection periods (2002–2026), and laboratory methods, so the pooled bound should be interpreted as a summary across heterogeneous surveillance settings rather than as a precise population parameter; the subgroup and leave-one-out sensitivity analyses were performed to probe this. A corollary is that the zero counts constrain but do not exclude low-frequency resistance: the pooled denominators give reasonable power to detect resistance at the 1 percent level for tetracycline and levofloxacin (n > 2,000, where even a single resistant isolate would have been recorded), but much lower power for the smaller drug-specific and subgroup analyses. In particular, the macrolide-susceptible comparison subgroup is small (n = 467 to 501, exact upper bounds 0.73 to 0.79 percent) and should be read as descriptive only: its zero count is compatible with a true resistance rate approaching 1 percent, and it cannot be used to predict second-line susceptibility in macrolide-susceptible isolates, whose resistance mechanisms are in any case independent of macrolide status. Second, the evidence is geographically concentrated (eight primary studies from China and three from Japan), limiting generalizability beyond East Asia. Third, the search was conducted in two tracks that were not executed on a single date: the Chinese-language databases were searched in September 2026, whereas the English/international databases reused the searches of the previous submission, supplemented by an updated PubMed re-run, because Web of Science and Scopus could not be re-run and Embase could not be searched for want of institutional access. Records indexed only in Embase, or English-language records published between the original English search and September 2026 in databases other than PubMed, may therefore have been missed; the two-track design is reported transparently in the Methods, and the prepared Embase strategy is retained for future updates. Fourth, interpretive criteria for second-line agents against *M. pneumoniae* are neither uniform nor fully established, and this is the most important constraint on the pooled estimates. The only organism-specific document, CLSI M43-A, provides provisional minimum inhibitory concentration breakpoints for a limited set of agents and none that formally define fluoroquinolone resistance; the included studies therefore applied heterogeneous criteria, ranging from CLSI M43-A (for example, Fang M 2026) to author-adapted thresholds derived from CLSI M100 (Guo D 2019) to no explicit susceptible/resistant classification at all. To avoid imposing a single contested breakpoint retrospectively and asymmetrically, we accepted each study's own susceptibility classification for the primary analysis and reported studies that provided only minimum inhibitory concentration distributions descriptively rather than pooling them. One such study, Wang Y 2016, reported levofloxacin MICs up to 4 µg/mL in two of 18 outbreak isolates, above the CLSI M43-A susceptible-only cutoff of 1 µg/mL; however, its own reference-susceptible strain M129 read 2 µg/mL, no quinolone-resistance breakpoint exists, and no *gyrA* or *parC* sequencing was reported, so these isolates could be neither confirmed as resistant nor dismissed, and the study was not pooled. A sensitivity analysis restricted to studies that used CLSI M43-A or an equivalent standardized criterion yielded the same zero-event finding. Accordingly, the pooled estimates should be read as resistance by each study's stated criteria, not as a single harmonized rate. Beyond breakpoint choice, the included studies also differed in testing medium (agar versus broth dilution), reference control strains, and incubation conditions, all of which affect absolute MIC values for this fastidious, cell-wall-deficient organism; these differences could in principle mask isolates that are susceptible but read near a breakpoint, although they are unlikely to overturn the uniform zero-resistance finding. Fifth, MRMP denominators vary widely, and the Morozumi M 2005 estimates rest on only 12 MRMP isolates. Sixth, a potential overlap of up to 24 Beijing isolates between Yan C 2025 and Yan C 2026 cannot be excluded; however, all analyses are zero-event, so such overlap cannot alter any pooled estimate, and the sensitivity analysis excluding Yan C 2025 left every conclusion unchanged. Seventh, the adapted quality checklist has not been formally validated. Eighth, the "resistance" percentages tabulated by He WQ 2025 were obtained from serology-diagnosed cases without any cultured isolate, reference method, or quality-control strain, and therefore cannot be interpreted as resistance rates; they were excluded from all quantitative synthesis and from Fig. 3. Ninth, the review evaluated *in vitro* susceptibility rather than clinical outcomes, so the results cannot establish comparative effectiveness, safety, or optimal timing of second-line therapy.

Future surveillance should be prospective, multicenter, and standardized. At a minimum, studies should use CLSI-based or clearly validated susceptibility methods, report MIC distributions rather than only categorical interpretations, provide separate pediatric MRMP denominators for each tested drug, and link phenotypic resistance to relevant molecular markers when available. Coordinated reporting across regions would allow earlier detection of emerging second-line resistance and would reduce the risk that isolated local signals are either overinterpreted or missed. Overall, the evidence is strongest where it matters most for this question, in sample size and in consistency: the uniform absence of second-line resistance across more than 2,000 cultured isolates, several countries, and two decades, with exact upper bounds at or below 0.17% for the two main classes. Its principal limitations are the non-uniform interpretive criteria across studies and the inherent property of zero-event data that a true, very low resistance rate cannot be precisely bounded; these are characteristics of surveillance evidence rather than deficiencies of the individual studies, which used standardized methods and were at low-to-moderate risk of bias.

Declarations

Ethics Approval and Consent to Participate

Not applicable. This is a systematic review and meta-analysis of published studies.

Consent for Publication

Not applicable.

Availability of Data and Materials

All data generated or analyzed during this study are included in this published article and its supplementary information files. The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing Interests

The authors declare that they have no competing interests.

Funding

This study was funded by the Beijing Nova Program (Grant No. 20230484439) and the Beijing High-Level Public Health Technical Personnel Construction Project (Grant No. 20230233). The funding bodies had no role in the design of the study, collection, analysis, and interpretation of data, or in writing the manuscript.

Authors' Contributions

Hao Chen: Methodology, data curation, formal analysis, writing - original draft. Deze Li: Data curation, validation, writing - review and editing. Chen Shen: Conceptualization, methodology, validation, interpretation of results, supervision, writing - review and editing. All authors read and approved the final manuscript.

Acknowledgements

Not applicable.

References

1. Waites KB, Xiao L, Liu Y et al (2017) *Mycoplasma pneumoniae* from the Respiratory Tract and Beyond. Clin Microbiol Rev 30(3):747–809. 10.1128/CMR.00114-16
2. Morozumi M, Takahashi T, Ubukata K (2010) Macrolide-resistant *Mycoplasma pneumoniae*: characteristics of isolates and clinical aspects of community-acquired pneumonia. J Infect Chemother 16(2):78–86. 10.1007/s10156-009-0021-4
3. Yan C, Sun H, Zhao H (2016) Latest surveillance data on *Mycoplasma pneumoniae* infections in children, suggesting a new epidemic occurring in Beijing. J Clin Microbiol 54(5):1400–1401. 10.1128/JCM.00184-16
4. Kim K, Jung S, Kim M, Park S, Yang H, Lee E (2022) Global trends in the proportion of macrolide-resistant *Mycoplasma pneumoniae* infections: a systematic review and meta-analysis. JAMA Netw Open 5(7):e2220949. 10.1001/jamanetworkopen.2022.20949
5. Wu PS, Chang LY, Lin HC et al (2013) Epidemiology and clinical manifestations of children with macrolide-resistant *Mycoplasma pneumoniae* pneumonia in Taiwan. Pediatr Pulmonol 48(9):904–911. 10.1002/ppul.22706
6. Lee H, Yun KW, Lee HJ et al (2018) Antimicrobial therapy of macrolide-resistant *Mycoplasma pneumoniae* pneumonia in children. Expert Rev Anti Infect Ther 16(1):23–34. 10.1080/14787210.2018.1414599
7. Jiang Y, Dou H, Xu B et al (2024) Macrolide resistance of *Mycoplasma pneumoniae* in several regions of China from 2013 to 2019. Epidemiol Infect 152:e75. 10.1017/S0950268824000323
8. Bradley JS, Byington CL, Shah SS et al (2011) The management of community-acquired pneumonia in infants and children older than 3 months of age: clinical practice guidelines by the Pediatric Infectious Diseases Society and the Infectious Diseases Society of America. Clin Infect Dis 53(7):e25–e76. 10.1093/cid/cir531
9. Page MJ, McKenzie JE, Bossuyt PM et al (2021) The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. BMJ 372:n71. 10.1136/bmj.n71
10. Sterne JAC, Sutton AJ, Ioannidis JPA et al (2011) Recommendations for examining and interpreting funnel plot asymmetry in meta-analyses of randomised controlled trials. BMJ 343:d4002. 10.1136/bmj.d4002
11. Page MJ, Higgins JPT, Sterne JAC et al (2024) Chapter 13: Assessing risk of bias due to missing evidence in a meta-analysis. In: Higgins JPT, Thomas J, Chandler J, Cumpston M, Li T, Page MJ, editors. Cochrane Handbook for Systematic Reviews of Interventions version 6.5. Cochrane; Available from: https://www.cochrane.org/authors/handbooks-and-manuals/handbook/current/chapter-13
12. Morozumi M, Hasegawa K, Kobayashi R et al (2005) Emergence of macrolide-resistant *Mycoplasma pneumoniae* with a 23S rRNA gene mutation. Antimicrob Agents Chemother 49(6):2302–2306. 10.1128/AAC.49.6.2302-2306.2005
13. Oishi T, Takahashi K, Wakabayashi S et al (2019) Comparing antimicrobial susceptibilities among *Mycoplasma pneumoniae* isolates from pediatric patients in Japan between two recent epidemic periods. Antimicrob Agents Chemother 63(7):e02517–e02518. 10.1128/AAC.02517-18
14. Guo DX, Hu WJ, Wei R et al (2019) Epidemiology and mechanism of drug resistance of *Mycoplasma pneumoniae* in Beijing, China: a multicenter study. Bosn J Basic Med Sci 19(3):288–296. 10.17305/bjbms.2019.4053
15. Wang Y, Ye Q, Yang D et al (2016) Study of two separate types of macrolide-resistant *Mycoplasma pneumoniae* outbreaks. Antimicrob Agents Chemother 60(7):4310–4314. 10.1128/AAC.00198-16
16. Wang N, Zhang H, Yin YH et al (2022) Antimicrobial susceptibility profiles and genetic characteristics of *Mycoplasma pneumoniae* in Shanghai, China, from 2017 to 2019. Infect Drug Resist 15:4443–4452. 10.2147/IDR.S370126

17. Oishi T, Yoshioka D, Nakano T et al (2022) Recent trend of antimicrobial susceptibility among *Mycoplasma pneumoniae* isolated from Japanese children. Microorganisms 10(12):2428. 10.3390/microorganisms10122428
18. Yan C, Chen Y, Ren X et al (2025) *In vitro* antimicrobial susceptibility of *Mycoplasma pneumoniae* isolates across different regions of China in 2023. JAC Antimicrob Resist 7(4):dlaf124. 10.1093/jacamr/dlaf124
19. Yan C, Chen Y, Su A et al (2026) Antimicrobial susceptibility and distribution characteristics of *Mycoplasma pneumoniae* isolates in Beijing, China, from 2017 to 2025. Pharmaceuticals (Basel) 19(3):488. 10.3390/ph19030488
20. Wu Q, Pan F, Wang N et al (2026) Genomic epidemiology and antimicrobial susceptibility of clinical *Mycoplasma pneumoniae* isolates in Shanghai, 2023–2024. Front Cell Infect Microbiol 16:1747629. 10.3389/fcimb.2026.1747629
21. Fang M, Wang X, Yu X et al (2026) Clonal replacement by a P1-1/ST3 lineage in pediatric *Mycoplasma pneumoniae*, Jinan, China, 2021–2024. Front Cell Infect Microbiol 15:1732239. 10.3389/fcimb.2025.1732239
22. Liu Y, Ye X, Zhang H et al (2010) Characterization of macrolide resistance in *Mycoplasma pneumoniae* isolated from children in Shanghai, China. Diagn Microbiol Infect Dis 67(4):355–358. 10.1016/j.diagmicrobio.2010.03.004
23. He WQ, Zhou J, Zhao QQ et al (2025) Clinical characteristics and drug resistance of *Mycoplasma pneumoniae* infection in children. Zhejiang Med J 47(19):2086–2090
24. Zhang D, Wang JJ, Lyu CX et al (2026) Molecular typing and drug resistance of *Mycoplasma pneumoniae* in children in Zibo, Shandong. J Mod Lab Med 41(1):116–120
25. Chen LJ, Li B, Yang LX (2025) Pathogen distribution in children with *Mycoplasma pneumoniae* pneumonia, influencing factors of concurrent liver damage, and predictive model construction. J Pathogen Biol 20(10):1266–1269
26. Liu X, Jiang Y, Chen X et al (2014) Drug resistance mechanisms of *Mycoplasma pneumoniae* to macrolide antibiotics. Biomed Res Int 2014:320801. 10.1155/2014/320801
27. Shi RH et al (2026) Molecular epidemiology and genomic characterization of postpandemic macrolide-resistant *Mycoplasma pneumoniae* in hospitalized children. Int J Infect Dis 169:108780. 10.1016/j.ijid.2026.108780
28. Morozumi M, Iwata S, Hasegawa K et al (2008) Increased macrolide resistance of *Mycoplasma pneumoniae* in pediatric patients with community-acquired pneumonia. Antimicrob Agents Chemother 52(1):348–350. 10.1128/AAC.00779-07
29. Ozaki T, Nishimura N, Ahn J et al (2007) Utility of a rapid diagnosis kit for *Mycoplasma pneumoniae* pneumonia in children, and the antimicrobial susceptibility of the isolates. J Infect Chemother 13(4):204–207. 10.1007/s10156-007-0519-6
30. Jia X, Chen Y, Gao Y et al (2024) Increased *in vitro* antimicrobial resistance of *Mycoplasma pneumoniae* isolates obtained from children in Beijing, China, in 2023. Front Cell Infect Microbiol 14:1478087. 10.3389/fcimb.2024.1478087[Excluded overlapping cohort; superseded by Yan C 2026 and not included in any analysis.]
31. Akaike H, Miyashita N, Kubo M et al (2012) Atypical Pathogen Study Group. *In vitro*activities of 11 antimicrobial agents against macrolide-resistant *Mycoplasma pneumoniae*isolates from pediatric patients: results from a multicenter surveillance study. Jpn J Infect Dis. ;65(6):535–538. 10.7883/yoken.65.535. [Excluded overlapping cohort with Oishi T 2019; data used in sensitivity analyses only.]
32. Wang N, Zhou Y, Zhang H et al (2020) *In vitro* activities of acetylmidecamycin and other antimicrobials against human macrolide-resistant *Mycoplasma pneumoniae* isolates. J Antimicrob Chemother 75(6):1513–1517. 10.1093/jac/dkaa027[Excluded overlapping cohort; subset of Wang N 2022.]
33. Kawai Y, Miyashita N, Kubo M et al (2013) Nationwide surveillance of macrolide-resistant *Mycoplasma pneumoniae* infection in pediatric patients. Antimicrob Agents Chemother 57(8):4046–4049 [Excluded overlapping cohort with Oishi T 2019; MIC90 data only.]
34. Liu Y, Zhang H, Ye XY et al (2009) [Susceptibility of *Mycoplasma pneumoniae* isolated in Shanghai from 2005 to 2008 to antimicrobial agents and the mechanism of macrolide resistance]. Chin J Microbiol Immunol 29(12):1110–1113. 10.3760/cma.j.issn.0254-5101.2009.12.015Chinese. [Excluded: overlapping cohort with no extractable second-line counts.]
35. Huang C, Yang J, Cao J et al (2025) Antimicrobial susceptibility and molecular characteristics of *Mycoplasma pneumoniae*isolates from children in Xi'an regions of China. Front Pediatr. ;13:1660687. 10.3389/fped.2025.1660687. [Excluded: commercial colorimetric kit not validated for *M. pneumoniae*susceptibility testing.]
36. Wang N, Chen Y, Qu X et al (2023) *In vitro* pharmacodynamics of nemonoxacin and other antimicrobial agents against *Mycoplasma pneumoniae*. Microbiol Spectr 11(6):e0243123. 10.1128/spectrum.02431-23
37. Morozumi M, Okada T, Tajima T et al (2017) Killing kinetics of minocycline, doxycycline and tosufloxacin against macrolide-resistant *Mycoplasma pneumoniae*. Int J Antimicrob Agents 50(2):255–257
38. Ishiguro N, Koseki N, Kaiho M et al (2017) Therapeutic efficacy of azithromycin, clarithromycin, minocycline and tosufloxacin against macrolide-resistant and macrolide-sensitive *Mycoplasma pneumoniae* pneumonia in pediatric patients. PLoS ONE 12(3):e0173635. 10.1371/journal.pone.0173635
39. Clinical and Laboratory Standards Institute (2011) Methods for Antimicrobial Susceptibility Testing for Human Mycoplasmas; Approved Guideline. CLSI document M43-A. Clinical and Laboratory Standards Institute, Wayne, PA
40. Xin D, Mi Z, Han X et al (2009) Molecular mechanisms of macrolide resistance in clinical isolates of *Mycoplasma pneumoniae* from China. Antimicrob Agents Chemother 53(5):2158–2159
41. Xie P, Liu J, Feng Y et al (2025) Evolution and characteristics of tetracycline resistance in *Mycoplasma pneumoniae*. Microbiol Spectr 13(7):e0339824. 10.1128/spectrum.03398-24
42. Liu GL, Luo YD, Yu YX (2026) Epidemiological characteristics and drug resistance of *Mycoplasma pneumoniae* in 2,753 children in the Wuzhou area. Med Lab Sci Clin 37(8):76–79

43. Zhao F, Liu G, Wu J et al (2013) Surveillance of macrolide-resistant *Mycoplasma pneumoniae* in Beijing, China, from 2008 to 2012. Antimicrob Agents Chemother 57(3):1521–1523
44. Yin YD, Wang R, Zhuo C et al (2017) Macrolide-resistant *Mycoplasma pneumoniae* prevalence and clinical aspects in adult patients with community-acquired pneumonia in China: a prospective multicenter surveillance study. J Thorac Dis 9(10):3774–3781
45. Pereyre S, Goret J, Bébéar C (2016) *Mycoplasma pneumoniae*: Current Knowledge on Macrolide Resistance and Treatment. Front Microbiol 7:974
46. Eshaghi A, Memari N, Tang P et al (2013) Macrolide-resistant *Mycoplasma pneumoniae* in humans, Ontario, Canada, 2010–2011. Emerg Infect Dis 19(9):1525–1527
47. Chua TP, Vodstrcil LA, Murray GL et al (2025) Evolving patterns of macrolide and fluoroquinolone resistance in *Mycoplasma genitalium*: an updated systematic review and meta-analysis. Lancet Microbe 6(7):101047
48. Lan S, Liu S, Cui W et al (2025) Emergence of Novel Fluoroquinolone Resistance Mutations in *Mycoplasma bovis*, China, 2008–2023. Emerg Infect Dis 31(8):1676–1679
49. Jain S, Williams DJ, Arnold SR et al (2015) Community-acquired pneumonia requiring hospitalization among U.S. children. N Engl J Med 372(9):835–845
50. Kutty PK, Jain S, Taylor TH et al (2019) *Mycoplasma pneumoniae* Among Children Hospitalized With Community-acquired Pneumonia. Clin Infect Dis 68(1):5–12
51. Waites KB, Talkington DF (2004) *Mycoplasma pneumoniae* and its role as a human pathogen. Clin Microbiol Rev 17(4):697–728
52. Gruson D, Pereyre S, Renaudin H et al (2005) *In vitro* development of resistance to six and four fluoroquinolones in *Mycoplasma pneumoniae* and *Mycoplasma hominis*, respectively. Antimicrob Agents Chemother 49(3):1190–1193
53. Bébéar CM, Pereyre S (2005) Mechanisms of drug resistance in *Mycoplasma pneumoniae*. Curr Drug Targets Infect Disord 5(3):263–271

Figures

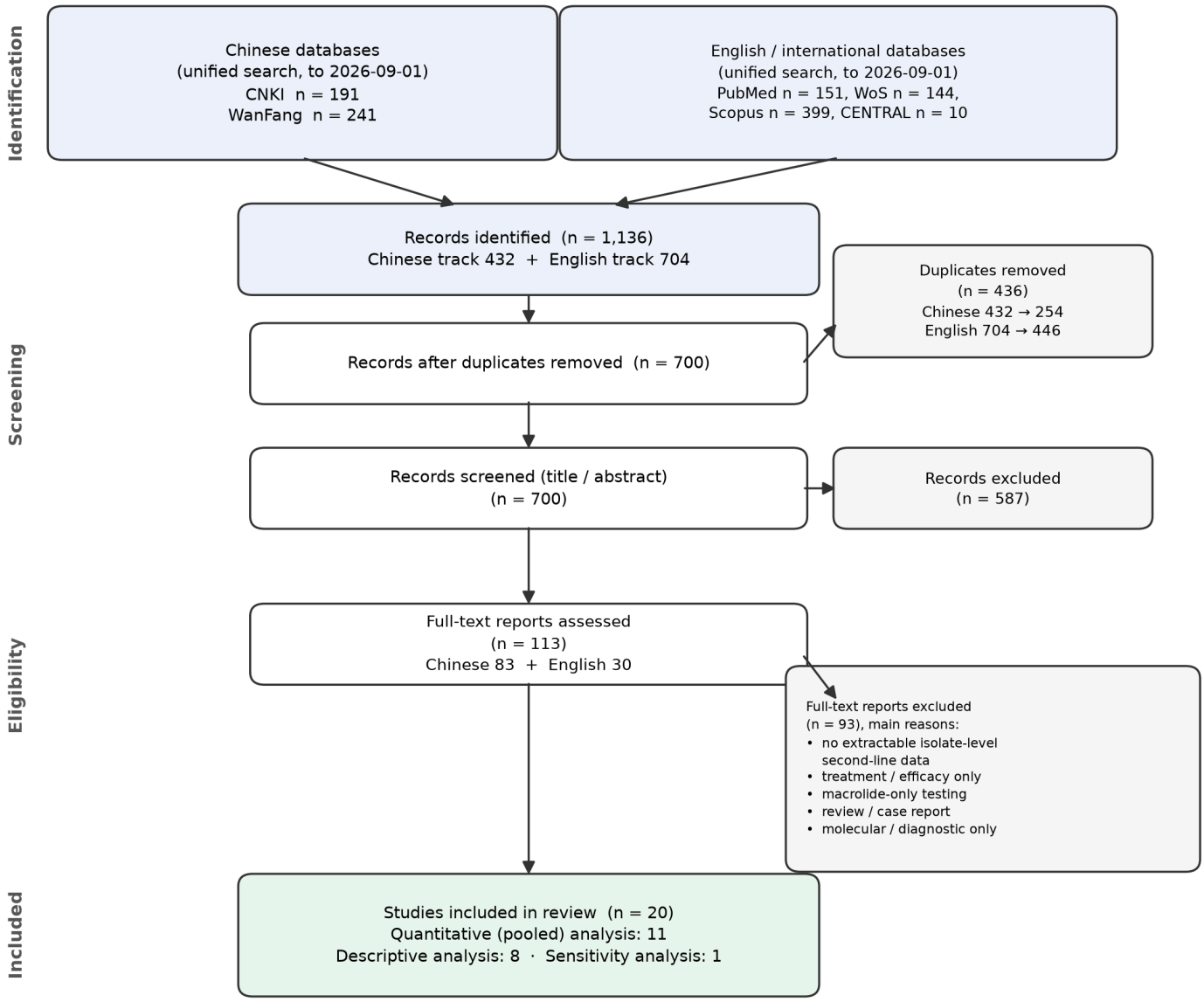


Figure 1

PRISMA 2020 flow diagram. A single unified Chinese-English mirror-image search of six databases to September 1, 2026 identified 1,136 records (Chinese track 432; English track 704). After deduplication (700 unique records) and title/abstract screening, 113 full-text reports were assessed and 20 studies were included (11 quantitative/pooled, 8 descriptive, 1 sensitivity).

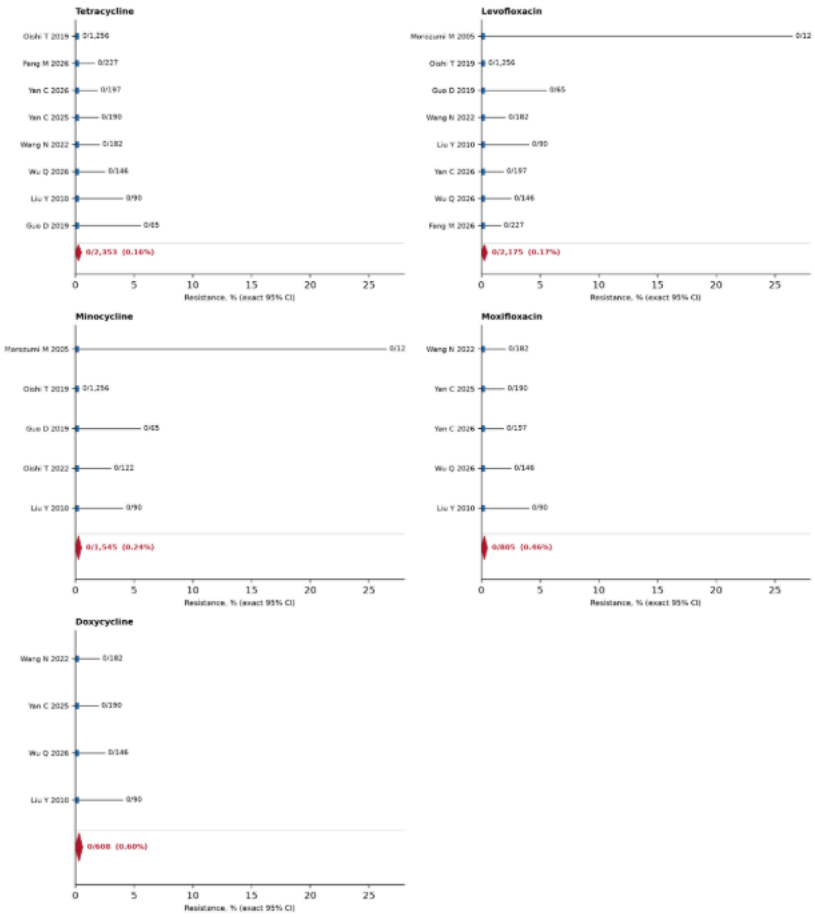


Figure 2

Forest plots of second-line antibiotic resistance among cultured pediatric *M. pneumoniae* isolates, by drug, on a common x-axis. Each row shows a study's resistant/tested count (blue square at 0%) with its exact 95% confidence interval; the red diamond is the pooled estimate. All events are zero, so confidence-interval width reflects sample size. Denominators are all cultured isolates classified by each study's own susceptibility criteria.

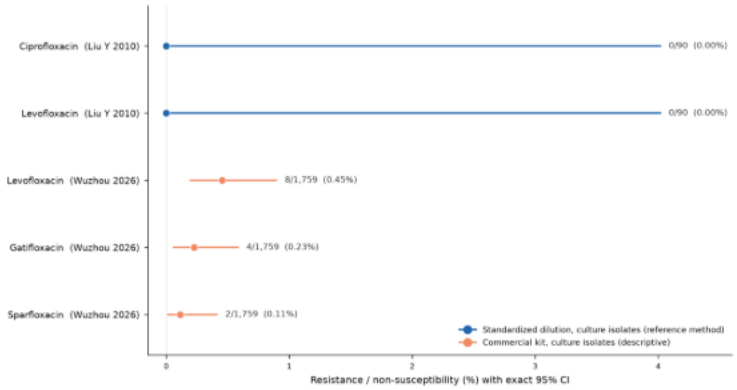


Figure 3

Second-line susceptibility by testing method among culture-confirmed isolates. Standardized broth-dilution data (reference method) and commercial-kit surveillance (descriptive) both show fluoroquinolone non-susceptibility below 0.5%; exact 95% confidence intervals are shown.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementaryMaterials.docx