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In-vitro antimicrobial activity of new antimicrobial agents against *Streptococcus pneumoniae* and potential resistance mechanisms: a multicenter study

Zichen Lei[1,2,3†], Qi Liu[1,2†], Yiqun Ma[2,4], Xinrui Yang[2,3], Hao Zu[5], Ziyao Li[2,3], Feilong Zhang[2,3], Dongya Pu[1,2], Yulin Zhang[2*] and Binghuai Lu[1,2,3,4*]

Abstract

Background *Streptococcus pneumoniae* is a major cause of invasive and non-invasive diseases, particularly in children and immunocompromised individuals, with an annual mortality of approximately 800,000 children worldwide. The rise of antibiotic-resistant strains complicates treatment, especially with increasing resistance to penicillin, macrolides, and fluoroquinolones. The study on the resistance of newly developed antimicrobial agents against *S. pneumoniae* was rarely reported. Furthermore, understanding the relationship between serotypes, resistance mechanisms, and virulence in *S. pneumoniae* is essential for disease management and vaccine development.

Methods A total of 208 *S. pneumoniae* isolates were collected across nine hospitals in seven Chinese cities/provinces from January 2023 to June 2024. Molecular characteristics were analyzed using whole-genome sequencing to identify serotypes, sequence types, virulence genes, and potential resistance mechanisms. Antibiotic susceptibility test (AST) was performed against 14 agents, involving new antibiotics (eravacycline, omadacycline, nemonoxacin, and contezolid).

Results Serotypes 19 F (24.6%) and 23 F (11.1%) predominated, with vaccine coverage rates of PCV13 at 66.8%. High resistance rates in *S. pneumoniae* were observed for erythromycin (208/208, 100%), clindamycin (197/208, 94.7%), and tetracycline (192/208, 92.3%). 13.5% (28/208) and 2.9% (6/208) strains were intermediate and resistant to penicillin, respectively. The new antibiotics showed low resistance, namely, 1.9% (4/208), 0.5% (1/208), 1.9% (4/208), and 7.2% (15/208) resistant to eravacycline, omadacycline, contezolid, and nemonoxacin, respectively. Resistance mechanisms included mutations in 23S rRNA for oxazolidinones, *tet* genes for tetracyclines, and *gyrA/parC* for fluoroquinolones.

†Zichen Lei and Qi Liu contributed equally to this work.

*Correspondence:
Yulin Zhang
zhangyulin07@163.com
Binghuai Lu
zs25041@126.com

Full list of author information is available at the end of the article

Conclusions *S. pneumoniae* in China exhibits high genetic diversity and significant antibiotic resistance, underscoring the need for continuous surveillance and updated vaccines. New antibiotics remain effective against multidrug-resistant strains, offering potential treatment options in clinical settings.

Highlights

- A multicenter epidemiological survey on *Streptococcus pneumoniae* in China.
- Resistance to 4 new antibiotics (eravacycline, omadacycline, nemonoxacin, and contezolid) was reported.
- Potential resistance mechanisms to tetracyclines, oxazolidinones, and fluoroquinolones were studied based on genomic analysis.

Keywords *Streptococcus pneumoniae*, Eravacycline, Omadacycline, Nemonoxacin, Contezolid, New antibiotics, Resistance

Introduction

Streptococcus pneumoniae is a frequent colonizer of the human nasopharynx with a colonization rate of 27–65% in children [1]. Both invasive pneumococcal diseases, including bacteremia and meningitis, and non-invasive pneumococcal diseases like pneumonia and otitis media can afflict individuals who are immunocompromised, children, or those with microbiota imbalances [2]. It presents as a burden associated with high morbidity and mortality globally. In 2019, a total of 829,000 fatalities globally were attributed to 11 distinct types of pneumococcal infection syndromes [3]. Capsular polysaccharide is one of the most important virulence factors in *S. pneumoniae*. Currently, more than 100 different capsular serotypes have been identified [4], which are capable of stimulating antibody-based immunity. Pneumococcal conjugate vaccines (PCVs) can dramatically reduce the incidence of pneumococcal diseases by targeting specific capsular polysaccharide of *S. pneumoniae* [5].

Antibiotics are widely used to treat bacterial infections. The growing resistance in *S. pneumoniae* strains to antibiotics like penicillin, macrolides, fluoroquinolones, and sulfamethoxazole-trimethoprim poses a serious challenge [6]. In severe pneumococcal diseases or those allergic to β-lactam antibiotics, the use of agents like vancomycin or alternative therapies targeting resistant strains may be indicated [7, 8]. Some newly developed antibiotics, including eravacycline, omadacycline, nemonoxacin, and contezolid, have demonstrated considerable efficacy in *S. pneumoniae* infections, particularly those caused by multidrug-resistant strains [9–11]. However, there remains a lack of testing data for these new antibiotics in China.

In this study, in-vitro antimicrobial activities of 7 categories of antimicrobial agents (β-lactam, fluoroquinolones, oxazolidinone, tetracycline, macrolides, lincosamides, and lipopeptide), including 4 above new antimicrobial agents, were assessed in *S. pneumoniae* isolated from 208 patients in China. In addition, the information on molecular serotypes, sequence typing (ST), virulence genes, and antibiotic resistance mechanisms of these strains is analyzed by whole-genome sequencing (WGS) technology.

This study aimed to (1) evaluate the in-vitro efficacy of new antibiotics (eravacycline, omadacycline, nemonoxacin, contezolid) against *S. pneumoniae* in China, (2) characterize the molecular epidemiology of strains (serotypes, STs, virulence genes), and (3) explore correlations between clonal lineages, serotypes, and resistance phenotypes to identify high-risk clones driving antimicrobial resistance.

Materials and methods

Study population and strains

This multicenter study was conducted across nine hospitals located in seven cities/provinces in China over a period spanning from January 2023 to June 2024. A total of 208 non-duplicate *S. pneumoniae* isolates were enrolled. Instances of multiple admissions for the same patient and strains isolated from the same individual were excluded from the study. This research received approval from the Ethics Committee of the China-Japan Friendship Hospital, and all procedures adhered to the principles in Declaration of Helsinki (2022-KY-054).

The *S. pneumoniae* isolates were recovered from different specimens, namely, sputum, bronchoalveolar lavage fluid (BALF), blood, secretions, pleural effusion, tissue and CSF. Therefore, the identification of all isolates was conducted based on characteristic colony morphology and optochin sensitivity tests, with confirmation achieved through matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS, Bruker Daltonics, Germany). The *S. pneumoniae* isolates were preserved in 25% sterile glycerol broth at -80 °C for subsequent analysis. Invasive pneumococcal disease (IPD) is defined as infection with *S. pneumoniae* identified in blood, cerebrospinal fluid, and/or any other sterile body fluid specimens, and a clinical diagnosis suggesting invasive disease [12].

WGS and bioinformatics analysis

Genomic DNA from strains was extracted using the QIAamp DNA Mini Kit (Qiagen). WGS was performed via Illumina NovaSeq PE150, with low-quality sequences and adaptors filtered out. Homology comparisons were performed using BLASTn (https://blast.ncbi.nlm.nih.gov/Blast.cgi) against the NCBI database. The genome of *S. pneumoniae* was annotated using Prokka v1.14.5 (https://github.com/tseemann/prokka). Phylogenetic analysis of high-quality SNPs involved Snippy v3.2-dev (https://github.com/tseemann/snippy), Gubbins v2.4.1 (https://github.com/nickjcroucher/gubbins), RAxMLv8.2.12 (GTR-GAMMA model) (https://github.com/amkozlov/raxml-ng), and iTOL v5.6 (https://itol.embl.de/upload.cgi) for visualization.

Pan-genome analysis with Roary [13] v3.11.2 (https://github.com/sanger-pathogens/Roary) categorized genes into 'core' (99–100% strains), 'soft core' (95–99%), 'shell' (15–95%), and 'cloud' (< 15%) groups using Prokka [14]-generated GFF3 files. Molecular serotyping was conducted with PneumoCaT v1.2.1 (https://github.com/ukhsa-collaboration/PneumoCaT), and MLST was based on seven housekeeping genes (*aroE*, *gdh*, *gki*, *recP*, *spi*, *xpt*, *ddl*) following PubMLST (https://pubmlst.org/) guidelines. Clonal complexes (CC) were defined by shared alleles, and relationships among STs and serotypes were visualized using the goeBURST algorithm in PHYLOViZ 2.0 (https://www.phyloviz.net/). Twelve virulence-related genes were identified using BLASTn, including *cps2A*, *lytA*, *pspA*, *cbpA*, *nanA*, *piaA*, *ply*, *psaA*, *pavA*, *spxB*, *htrA*, and *clpP*. All genes were compared with ATCC 49619 as a reference.

In-vitro antimicrobial susceptibility testing (AST)

AST of all 208 isolates was determined by the manual microdilution broth method (bio-KONT, Ltd. China). The antibiotics used in this study included penicillin, ceftriaxone, ertapenem, levofloxacin, linezolid, tetracycline, tigecycline, daptomycin, clindamycin and erythromycin, and new antibiotics eravacycline, omadacycline, nemonoxacin, and contezolid. All susceptibility tests and results interpretations were performed according to the guidelines and criteria established by the Clinical and Laboratory Standard Institute (CLSI) 2024 [15]. The quality-control strain was *S. pneumoniae* ATCC 49619, which was included in each set of tests to ensure the reliability of the results. Isolates of *S. pneumoniae* were categorized as multidrug-resistant (MDR) if exhibiting resistance to ≥ 3 classes of antibiotics. New antimicrobial agents are defined as those discovered and brought to market within recent years, including nemonoxacin (fluoroquinolones), contezolid (oxazolidinone), eravacycline (tetracycline) and omadacycline (tetracycline).

Data analysis

Baseline data were described using standard deviation for normally distributed data. Comparative analysis utilized the chi-square test for categorical variables and the independent sample t-test for continuous variables. All data were analyzed using SPSS 22.0, with $p < 0.05$ considered statistically significant.

Results

Characteristics of pneumococcal isolates

In this study, a collection of 208 *S. pneumoniae* isolates from 9 hospitals were collected. The demographic and clinical characteristics of 208 patients were outlined in Fig. 1A/B. The ratio of male to female patients was 1.8 (133 males to 75 females), with the median age being 8 years (range: 1 month to 95 years). Lower respiratory specimens were the most specimen type (141/208, 67.8%), including sputum and BALF. Based on the sample types and the site of strain isolation, invasive infections accounted for 26.0% of the cases.

Molecular epidemiology

Among the 208 *S. pneumoniae* isolates analyzed, the predominant serotype identified was 19F (49 strains, 23.6%), followed by 23F (22, 10.6%), 3 (16, 7.7%), 14 (12, 5.8%), and 6B (12, 5.8%), respectively, as shown in Fig. 1C. Overall vaccine coverage for PCV7, PCV10 and PCV13 serotypes was 49.2%, 50.8% and 66.8%, respectively. A total of 79 individual STs were distinguished in 208 isolates of *S. pneumoniae*, and the predominant STs were ST271 (16.6%) and ST320 (4.8%).

Clonal conformation clustering analysis of the strains revealed that the predominant clonal complex (CC) was CC271, including ST271 and ST320. A correlation analysis performed between the STs and serotypes demonstrated a propensity for strains sharing the same serotype to cluster together. Notably, CC271 displayed a strong association with serotypes 19 F and 19 A, particularly with ST271-19 F and ST320-19 A. CC271 strains (32.6% of isolates) exhibited significantly higher resistance rates compared to non-CC271 strains, suggesting clonal expansion of multidrug-resistant lineages.

The distribution of virulence genes is detailed in Fig. 2, which uses different colors to illustrate the proportion of alignment between strains and reference sequences. The majority of isolates typically possess common virulence genes, such as *cbpA*, *clpP*, *pavA*, *psaA*, *spxB* (each constituting 97.0%), *lytA* (93.0%), *nanA* (90.0%), and *ply* (90.0%). Moreover, around 86.0% of the isolates harbor *pspA*, while 81.0% carry *piaA*. Notably, the distribution of virulence genes exhibited a significant correlation with CC. The carriage rate of all virulence factors in the CC271 group was 100%, markedly higher than that in the non-CC271 group.

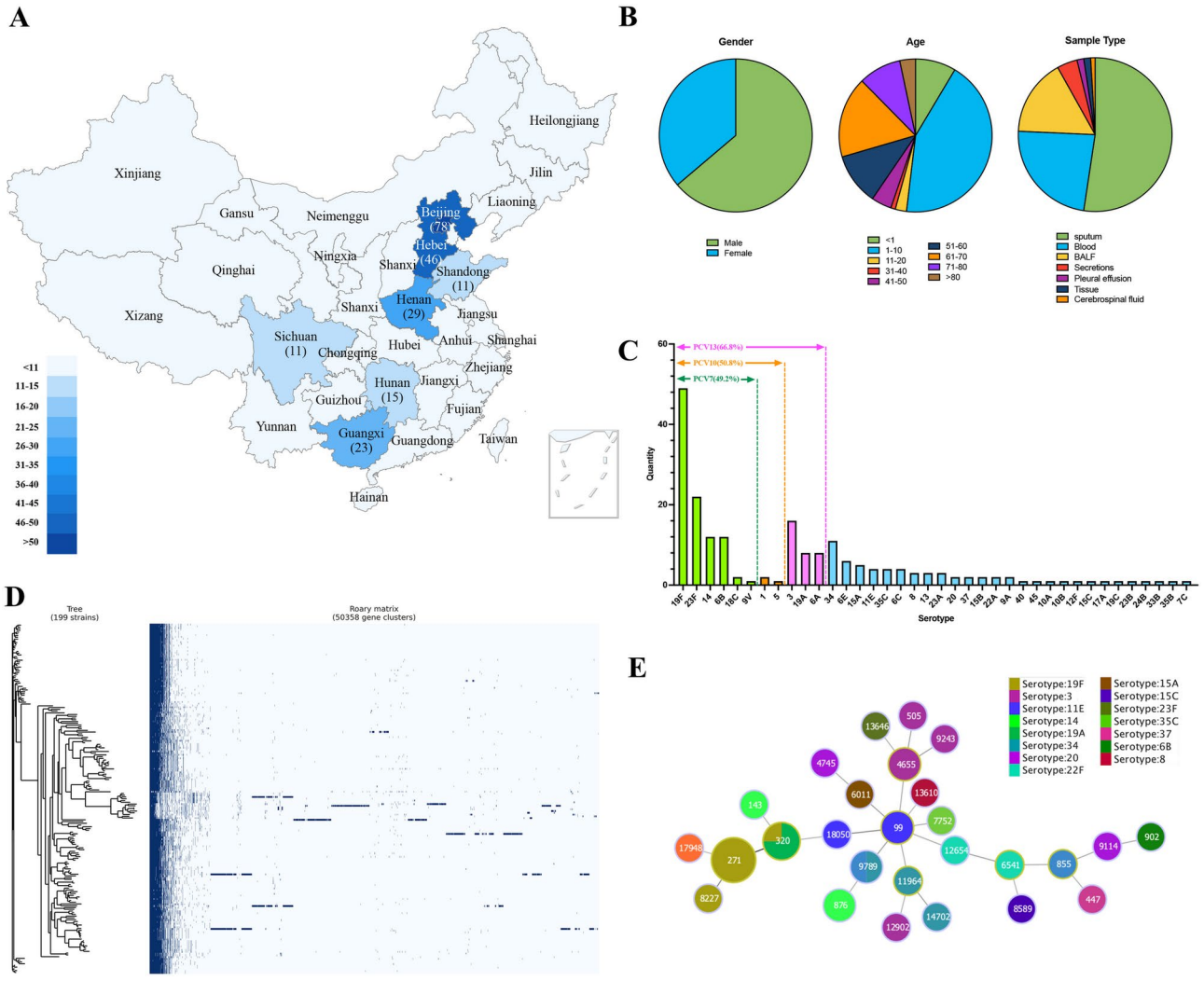

Fig. 1 Geographic distribution, demographics, serotypes, and genetic diversity of *Streptococcus pneumoniae* isolates. **(A)** Geographic distribution of 208 isolates across seven provinces in China. **(B)** Patient demographics by gender, age, and sample type. **(C)** Serotype distribution. **(D)** Phylogenetic tree and pan-genome analysis. **(E)** Minimum spanning tree of sequence types

As depicted in Fig. 1D, based on the pan-genome analysis using Roary, the dataset comprised 208 *S. pneumoniae* strains, revealing a total of 50,358 gene clusters. The distribution of genes across genomes showed a highly skewed pattern, with the majority of genes being unique to individual strains, as depicted in the histogram. The core genome, shared by 99% of the strains, consisted of 414 genes, while the accessory genome included 2,075 shell genes and 47,519 cloud genes. The phylogenetic tree and corresponding Roary matrix illustrate the genetic diversity among the strains, highlighting the presence of both conserved and highly variable regions.

Antibiotic resistance distribution and multidrug-resistant strains

AST was carried out on 14 antibiotics, with their results detailed in Fig. 3; Table 1. According to the CLSI guidelines, 208 (100.0%), 197 (94.7%), and 192 (92.3%) of 208 *S. pneumoniae* strains exhibited resistance to erythromycin, clindamycin and tetracycline, respectively. The resistance rates for other antibiotics remained low, namely, tigecycline (17/208, 8.2%), penicillin (6/208, 2.9%), ceftriaxone (14/208, 6.7%), ertapenem (2/208, 1.0%), levofloxacin (13/208, 6.3%) and daptomycin (9/208, 4.3%). They also exhibit relatively low resistance rates to new antibiotics, namely, nemonoxacin (15/208, 7.2%), eravacycline (4/208, 1.9%), omadacycline (1/208, 0.5%), and contezolid (4/208, 1.9%). The distribution of MICs of 14 antibiotics against 208 *S. pneumoniae* strains across 14 antibiotics is illustrated *via* violin plots in Fig. 3A. Subsequently, we quantified the number of antibiotic classes to which each strain exhibited resistance, as shown in Fig. 3B. A significant majority of the strains (162/208, 77.9%) demonstrated resistance to four

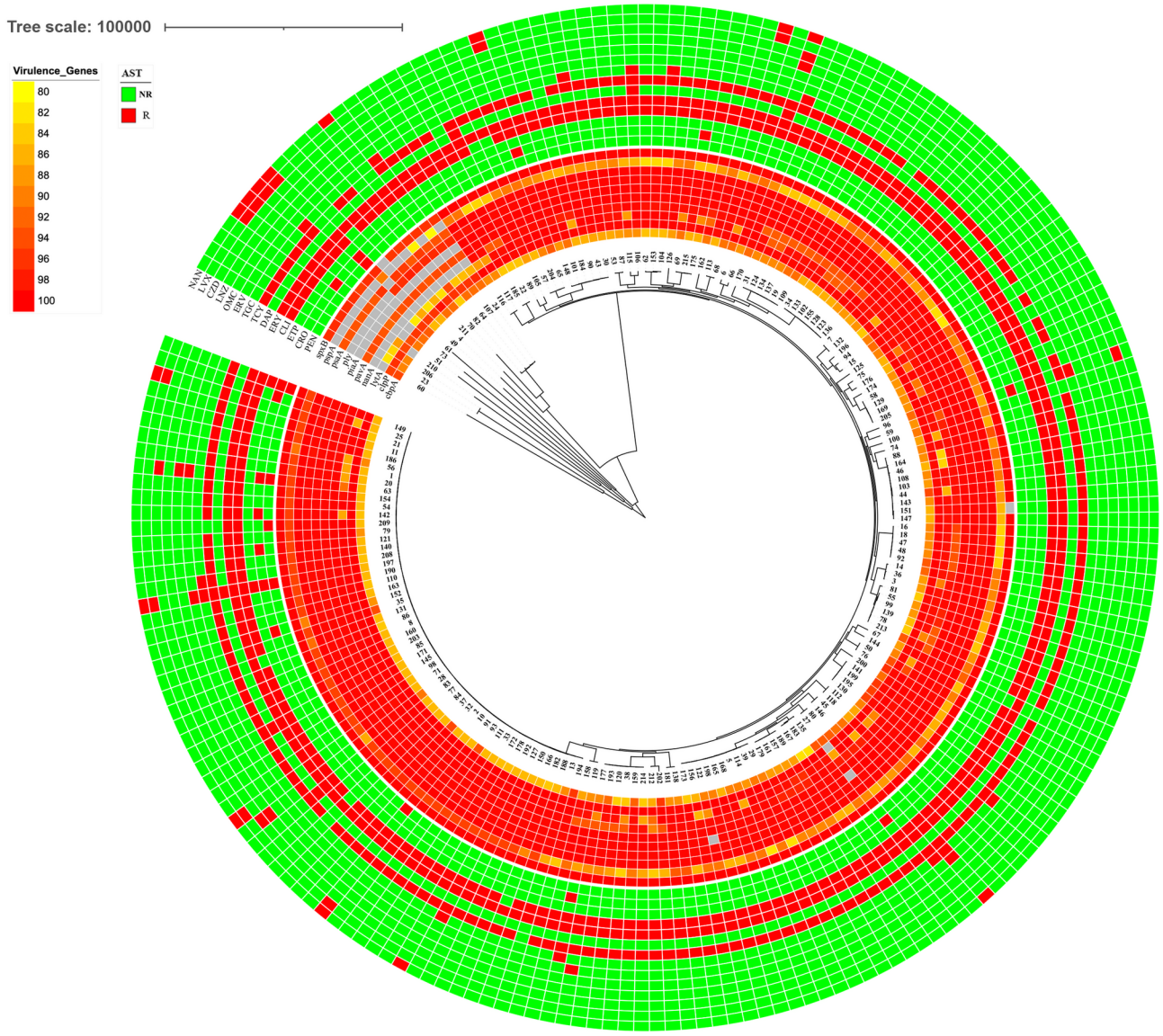

Fig. 2 Phylogenetic relationships, serotype distribution, resistance, and clinical outcomes of Streptococcus pneumoniae isolates. A phylogenetic tree of 208 *Streptococcus pneumoniae* isolates is displayed alongside metadata on sample type, geographic location, age, serotype, sequence type (MLST), antibiotic resistance, and clinical outcomes

or fewer antibiotics, predominantly clindamycin, erythromycin, tetracycline, and tigecycline, with the first three being the most prevalent. Notably, four strains exhibited exceptionally high levels of resistance, specifically to 12, 11, 9, and 9 antibiotics, respectively. The occurrence of multidrug resistance among these strains is uncommon.

Subsequently, we analyzed the resistance rates across various groups, categorized by the nature of the infection (invasive versus non-invasive) and by age (adult versus child), as illustrated in Fig. 3C and D. Except for a limited number of antibiotics, the resistance rates for the majority of antibiotic types in adults were found to be greater than those observed in children. Furthermore, the resistance rate linked to non-invasive infections was found to be higher than that associated with invasive infections, with a statistical significance of $P < 0.05$.

New antibiotic resistance mechanisms

Four strains exhibiting resistance to oxazolidinone antibiotics, specifically linezolid and contezolid, were identified, as illustrated in Fig. 4A. A comparative analysis of the resistance loci revealed point mutations at positions C2163A, A2203G, G2211T, and A2734C within the secondary structure of the peptidyl transferase central loop of the 23S rRNA domain V. The MIC of the mutant strains for linezolid was observed to increase by more than 4-fold compared to the control group. However, *cfr*

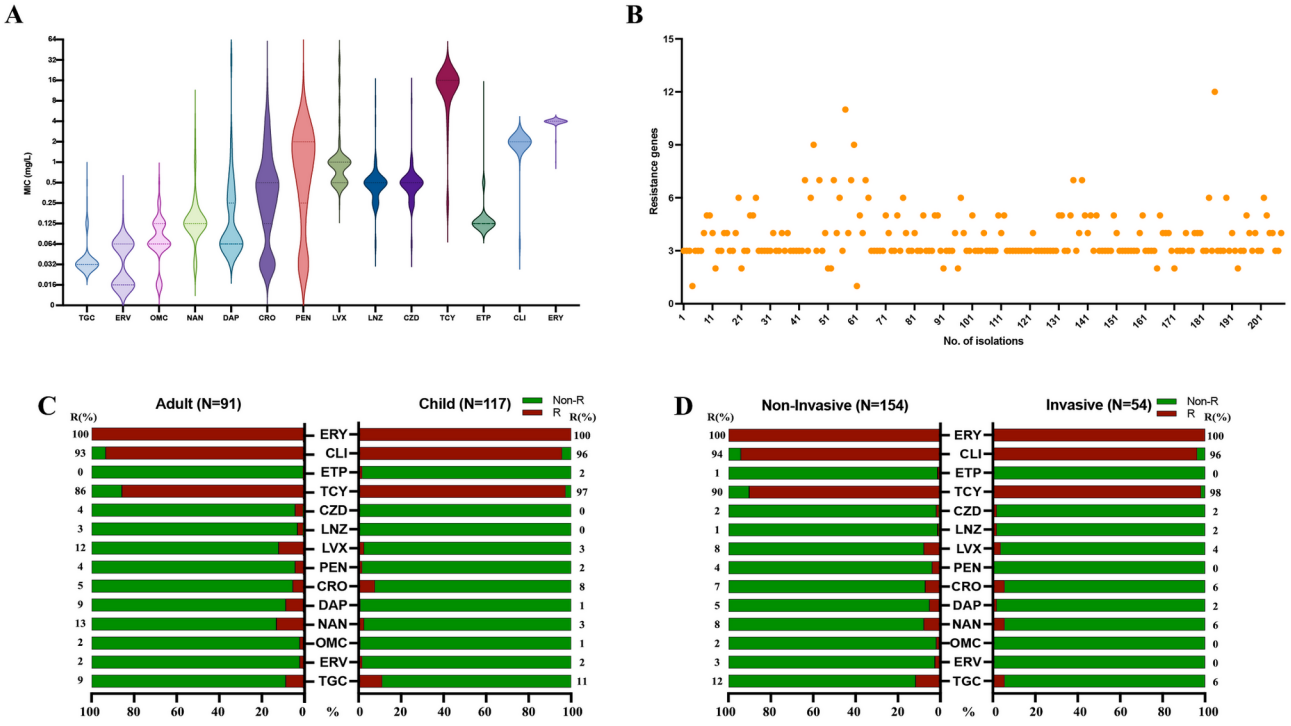

Fig. 3 Antibiotic susceptibility and resistance profiles of *Streptococcus pneumoniae* isolates. **(A)** Violin plots illustrating MIC distributions for all tested antibiotics. **(B)** Scatter plot depicting the number of resistance phenotypes per isolate. **(C)** Comparison of resistance rates between adult and pediatric isolates. **(D)** Comparison of resistance rates between invasive and non-invasive infections

and *optrA* genes, typically associated with oxazolidinone resistance, were not detected in these strains.

A total of 17 strains were identified as resistant to ≥1 of three tetracycline derivatives: tigecycline (17), eravacycline (4), and omadacycline (1), and all carried *tet* gene, as depicted in Fig. 4B. Furthermore, two of these strains exhibited point mutations within the *tet* gene, while one strain was found to possess multiple copies of the gene. Among the strains analyzed, one strain displayed resistance to both eravacycline and omadacycline, and three strains displayed resistance to eravacycline. They were noted to contain new mutation sites in the resistance genes associated with this phenotype, specifically *rpsJ* and *rpsC*.

As shown in Fig. 4C, 15 S. *pneumoniae* strains exhibited resistance to fluoroquinolones, specifically levofloxacin and nemonoxacin. The analysis of 6 prevalent resistance genes, namely, *gyrA*, *gyrB*, *parC*, *parE*, *patA*, and *patB*, indicated that all 15 strains possessed point mutations, with sequence homology ranging from 79.0 to 99.0%, and the number of mutation sites ranging from 2 to 431. Notably, these four strains displayed a significant number of mutant genes and sites, which correlated with their elevated MIC for fluoroquinolones. Additionally, other strains also demonstrated a relationship between their resistance phenotypes and mutations in resistance genes. Our results showed that the mutations of the above genes will confer resistance to both old and new antibiotics.

Discussion

This study investigated the epidemiology of *S. pneumoniae* in China, particularly capsular serotypes and virulence, along with the in-vitro antimicrobial activity of some newly developed antibiotics, including eravacycline, omadacycline, nemonoxacin, and contezolid against the microorganism.

Serotype is the most important epidemiological marker and vaccine target in *S. pneumoniae*, since this may differ both geographically and temporally [16]. In this study, serotype analysis of *S. pneumoniae* showed serotypes 19 F and 23 F were predominant, in consistent with global trends observed in invasive pneumococcal diseases [17]. However, analysis of vaccine coverage rates for PCV7, PCV10, and PCV13 suggested that many circulating strains are not covered, highlighting the need for updated vaccines. The presence of non-typeable strains points to gaps in vaccine efficacy, emphasizing the importance of continuous surveillance to refine vaccine strategies. MLST analysis uncovered substantial genetic diversity, including the identification of new genotypes. The predominance of ST271, related to serotypes 19 F and 19 A, indicated clonal spread driving the persistence of certain serotypes despite vaccination. A

Table 1 The in-vitro activities of 14 novel antimicrobial agents against 208 *S. pneumoniae* strains

Antibiotic	Breakpoints	MIC (mg/L)											MIC_{50}	MIC_{90}	Ratio (*N* = 208)	
		< 0.064	**0.064**	**0.125**	**0.25**	**0.5**	**1**	**2**	**4**	**8**	**16**	**> 16**			**Non-*R* (%)**	***R* (%)**
PEN (Parenteral (nonmeningits))	≥ 8		42	9	13	23	40	47	**28**	2	4		1	4	202 (97.1)	6 (2.9)
PEN (Parenteral (meningitis))	≥ 0.12														42 (20.2)	166 (79.8)
PEN (Oral penicillin V)	≥ 2														127 (61.1)	81 (38.9)
CRO	≥ 4		49	19	37	50	20	**19**	9	2	3		0.25	2	194 (93.3)	14 (6.7)
ETP	≥ 4				185	14	3	**4**	1	1			0.25	0.25	206 (99.0)	2 (1.0)
CLI	≥ 1			8	2	**1**	3	194					2	2	11 (5.3)	197 (94.7)
ERY	≥ 1						2	5	201				4	4	0 (0.0)	208 (100.0)
DAP	≥ 2		124	1	39	27	8	6	1		2		0.064	0.5	199 (95.7)	9 (4.3)
TCY	≥ 4					11	1	**4**	8	18	166		16	16	16 (7.7)	192 (92.3)
TGC	≥ 0.125		191	15	1	1							0.064	0.125	191 (91.8)	17 (8.2)
ERV	≥ 0.125	105	99	3	1								< 0.064	0.064	204 (98.1)	4 (1.9)
OMC	≥ 0.5	27	109	59	**12**	1							0.064	0.125	207 (99.5)	1 (0.5)
LNZ	≥ 2			5	42	144	13		2	2			0.5	0.5	204 (98.1)	4 (1.9)
CZD	≥ 2			5	36	149	14		1	3			0.5	0.5	204 (98.1)	4 (1.9)
LVX	≥ 8				2	78	104	8	**3**	4	5	4	1	2	195 (93.7)	13 (6.3)
NAN	≥ 1		19	152	20	2	8	4	3				0.125	0.25	193 (92.8)	15 (7.2)

* *Notes* The values in bold indicate intermediate. The values in bold and underlined indicate resistance. TGC: Tigecycline, ERV: Eravacycline, OMC: Omadacycline, NAN: Nemonoxacin, DAP: Daptomycin, CRO: Ceftriaxone, PEN: Penicillin, LVX: Levofloxacin, LNZ: linezolid, CZD: Contezolid, TCY: Tetracycline, ETP: Ertapenem, CLI: Clindamycin, ERY: Erythromycin. $MIC_{50/90}$, minimum inhibitory concentration at which 50/90% of isolates were inhibited. R: Resistance, Non-R: Non-Resistance. Breakpoints are based on CLSI 2024 guidelines.

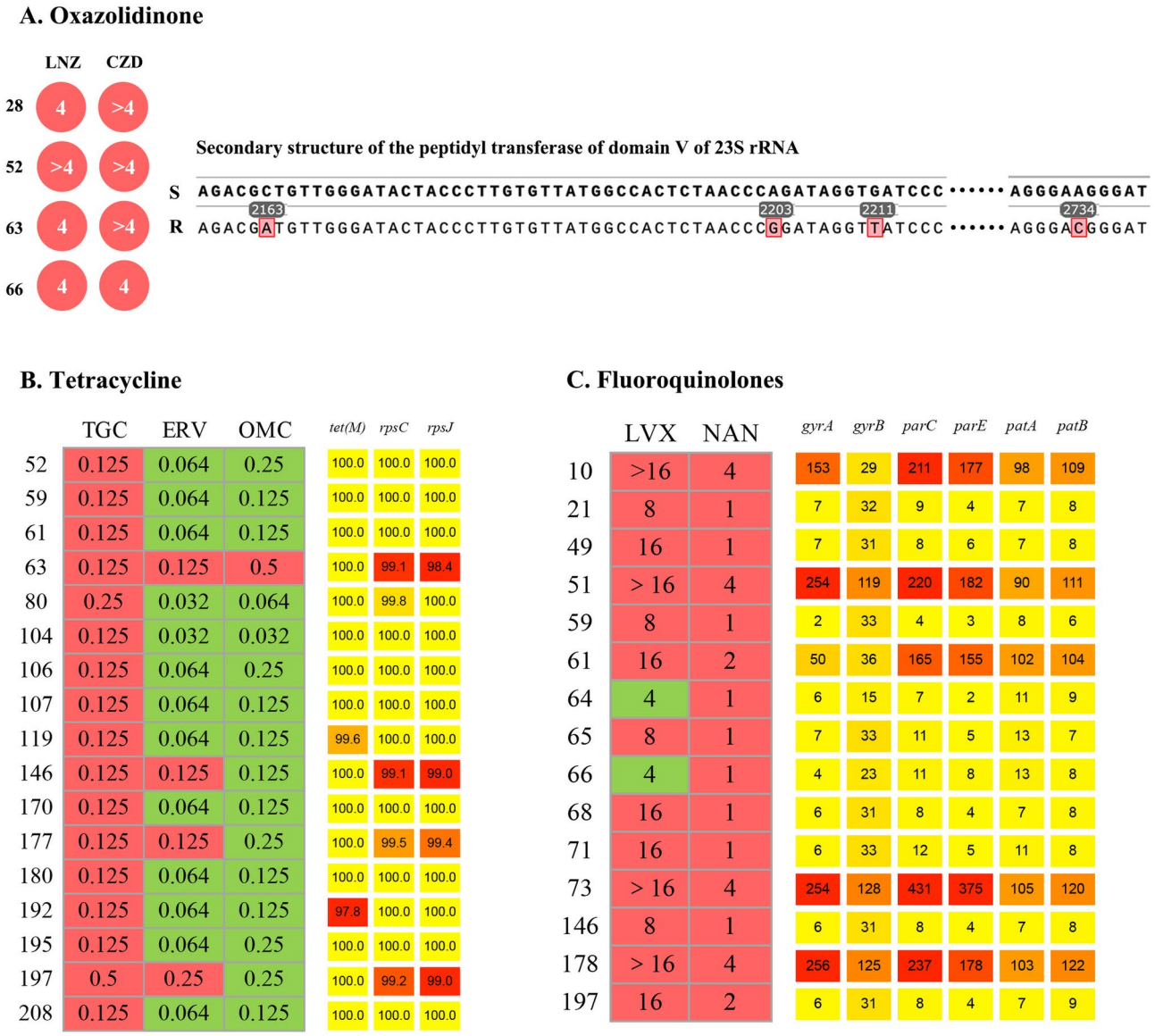

Fig. 4 Resistance mechanisms of *Streptococcus pneumoniae* to oxazolidinones, tetracyclines, and fluoroquinolones. The first column before the antibiotics represents the strain identification numbers. **(A)** Resistance to oxazolidinones (linezolid, contezolid) linked to mutations in the 23S rRNA central loop of domain V. **(B)** Resistance to tetracyclines (tigecycline, eravacycline, omadacycline) associated with the presence of *tet*(M) and mutations in *rpsC* and *rpsJ*. Each percentage under the columns with gene names indicates the percentage of sequence identity of the gene compared to the reference strain. **(C)** Fluoroquinolone resistance (levofloxacin, nemonoxacin) linked to mutations in *gyrA*, *gyrB*, *parC*, *parE*, *patA*, and *patB* genes. The numbers under the gene name columns represent the number of mutations (mismatches or deletions) in the gene compared to the reference strain

strong correlation between clonal complexes, particularly CC271, and serotypes, especially 19 F and 19 A, underscores the role of genetic factors in *S. pneumoniae* epidemiology, influencing transmission patterns and vaccine escape, with CC271 showing a higher carriage rate of virulence factors.

Notably, the predominance of serotype 19 F/CC271 in this study is consistent with reports from Taiwan, China [18]. However, unlike European studies where non-vaccine serotypes [3] (e.g., 8 and 12 F) have become dominant following the introduction of PCV13, the persistence of vaccine-targeted serotypes (19 F, 23 F) in China likely results from delayed PCV13 adoption and its low coverage. This highlights the urgent need for expansion of vaccination programs to effectively control clonal transmission of resistant strains.

The virulence of *S. pneumoniae* is conferred by capsular polysaccharides and various virulence factors, which may vary among different clades. Understanding the virulence profile of isolates is crucial for predicting disease severity and outcomes of infections, and it allows for risk assessment during the early stages of the disease [19]. The

distribution of virulence genes supports the clonal nature of infections, particularly within CC271, which carries more virulence factors. This implies an enhanced pathogenic potential in certain clonal complexes, contributing to their epidemiological success. The widespread presence of key virulence genes across isolates highlights the necessity for targeted intervention strategies. WGS analysis reveals the extensive genetic diversity, with a large accessory genome contributing to species adaptability. The discovery of unique genes suggests that horizontal gene transfer significantly diversifies pneumococcal populations, enabling the emergence of new strains with increased virulence or resistance [20].

AST revealed alarming resistance rates to commonly used antibiotics such as erythromycin, clindamycin, and tetracycline. Concurrently, the widespread use of penicillin has resulted in a notable rise in the prevalence of penicillin resistance [6]. Some new antibiotics, including eravacycline, omadacycline, nemonoxacin, and contezolid, demonstrate considerable antimicrobial efficacy in *S. pneumoniae*. Therefore, they possess the potential to serve as a therapeutic alternative.

Contezolid, similar to linezolid but incorporating a modified substituent on the aromatic ring, is a member of the oxazolidinone class of antibiotics, showing in-vitro efficacy against many Gram-positive cocci. A large-scale global study assessed the effectiveness of linezolid against *S. pneumoniae* (6691 isolates) during 2004–2012, with a 100% susceptibility [21], while another meta-analysis demonstrated that the prevalence of linezolid resistance fluctuated between 0% and 4.86% in *S. pneumoniae* isolates in 2014–2024 [22], as similar to our study (1.9%). Further, we observed that linezolid and contezolid exhibited the same resistance phenotypes, with their MIC values also being comparable. Previous research indicated that mutations G2576T and A2503G [23, 24] in the peptidyl transferase central loop of the ribosomal 23S rRNA domain V are linked to linezolid resistance in *S. pneumoniae*. In our study, mutations were identified at positions C2163A, A2203G, G2211T, and A2734C, respectively. Mutations occurring in the central loop of the 23S rRNA domain V, as well as in ribosomal proteins L3 and L4, are predominantly attributed to prolonged exposure to linezolid. Additionally, *cfr* [25] and *optrA* [26] have been confirmed to confer non-mutational resistance to linezolid in other bacterial species, as unidentified in our linezolid-resistant strains.

The three tetracycline derivatives, namely, tigecycline, eravacycline, and omadacycline, shared similar chemical structures, exhibiting reversible binding to the decoding center of the 30S ribosomal subunit [27]. Among the strains exhibiting resistance to the three tetracycline derivatives, only a limited number of strains of eravacycline and omadacycline exhibited resistance comparable to those of tigecycline. Furthermore, the MIC values of the three antibiotics were comparable. However, the resistance breakpoint for tigecycline was higher than those of eravacycline and omadacycline. Therefore, if resistance to eravacycline or omadacycline is detected, it can be inferred that tigecycline is resistant. Resistance to tigecycline among *S. pneumoniae* remains relatively low in a study in India (2015–2017) [28]. Between 2004 and 2010, the resistance rate of *S. pneumoniae* isolates to tigecycline in the Asia-Pacific region was documented at 2% [29]. However, the sensitivity of eravacycline and omadacycline in *S. pneumoniae* was rarely documented. In our study, the resistance rates of *S. pneumoniae* to tigecycline, eravacycline, and omadacycline were 10.1%, 1.9%, and 1.4%, respectively. This can be explained by structural modifications that enhance target binding and reduce recognition by common tetracycline resistance mechanisms. In vitro studies have indicated that Tet proteins, including Tet(X), Tet(A), Tet(K), and Tet(M) [30], may undergo mutations that confer reduced sensitivity to tigecycline. Furthermore, research on *S. pneumoniae* has investigated the implications of mutations in *rpsJ*, 16S rRNA and *rpsC* [31]. The introduction of the *rpsJ* G178T mutation into *S. pneumoniae* R6 resulted in a fourfold increase in resistance to both tigecycline and tetracycline [32]. Similarly, the introduction of the *rpsC* allele was associated with decreased susceptibility to tigecycline. In our research, all tigecycline-resistant strains were found to carry *tet* genes, which included multiple copies and point mutations. Additionally, novel mutation sites were identified in the *rpsJ* and *rpsC* genes.

Fluoroquinolones are a category of synthetic broad-spectrum antibiotics that function by inhibiting bacterial DNA synthesis through targeting DNA gyrase (*gyrA*) and topoisomerase IV (*parC*). While the global prevalence of fluoroquinolone-resistant *S. pneumoniae* remains relatively low (≤ 1%), the dissemination of resistant clones has led to an increasing prevalence in certain regions [33]. In our research, the MIC values for two fluoroquinolones, levofloxacin and nemonoxacin, exhibited considerable variation. While both agents demonstrated resistance concurrently, nemonoxacin had a substantially lower MIC. This can be explained by that nemonoxacin has a distinct arrangement of substituents and ring systems within the quinolone core. Resistance to fluoroquinolone mainly develops from spontaneous mutations in the quinolone resistance-determining region (QRDR) of topoisomerase IV and DNA gyrase genes, particularly in *parC* and *gyrA*, with occasional mutations in *parE* and *gyrB* contributing to low-level resistance. Overexpression of efflux systems could also play a role, typically causing modest resistance but facilitating QRDR mutations. Taken together, efflux activity and initial mutations can substantially elevate the MIC. The major facilitator

pmrA was the first characterized efflux system, and ATP-binding cassette transporters *patA* and *patB* function as heterodimers, conferring broad antimicrobial resistance [34]. In the present study, 15 strains exhibited resistance to fluoroquinolones, resulting in a resistance rate of 7%. An examination of six above prevalent resistance genes revealed that all strains possessed point mutations, with sequence homology varying between 79% and 99%. This suggests a high degree of mutability at the loci associated with fluoroquinolone resistance genes.

In conclusion, our study highlights the complex interplay of genetic, epidemiological, and clinical factors influencing *S. pneumoniae* infections in China. Our results revealed resistance mechanisms against novel antibiotics involved point mutations, gene deletions, and phenotype alterations. The low incidence of resistance has significant implications for managing multidrug-resistant *S. pneumoniae* infections.

Author contributions

Project design: LBH, LZC and LQ. Methodology: LZC, LZY and YXR. Project administration: LBH and ZYL. Data analyses: LZC, MYQ, ZFL, PDY and ZH. Manuscript writing: LZC and LQ. All authors provided final approval of the version submitted for publication.

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Data availability

The datasets generated and analysed during the current study are available in the NCBI BioProject repository, accession number: PRJNA1207438.

Declarations

Ethical approval

Given the retrospective nature of this cohort study using anonymous clinical data, the Ethics Committee of China-Japan Friendship Hospital (approval No. 2022-KY-054) granted ethical approval with a waiver of informed consent for the use of medical records and *S. pneumoniae* strains.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

Author details

[1]China-Japan Friendship Institute of Clinical Medical Sciences, China-Japan Friendship Hospital, Beijing, China
[2]Laboratory of Clinical Microbiology and Infectious Diseases, Department of Pulmonary and Critical Care Medicine, Center of Respiratory Medicine, Beijing Key Laboratory of Surveillance, Early Warning and Pathogen Research on Emerging Infectious Diseases, National Center for Respiratory Medicine, China-Japan Friendship Hospital, Beijing, China
[3]Peking Union Medical College, Chinese Academy of Medical Sciences, Beijing, China
[4]Peking University China-Japan Friendship School of Clinical Medicine, Beijing, China
[5]Capital Medical University-YanJing Medical School, Beijing, China

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References

1. Weiser JN, Ferreira DM, Paton JC. Streptococcus pneumoniae: transmission, colonization and invasion. Nat Rev Microbiol. 2018;16(6):355–67.
2. Estimates of the. global, regional, and National morbidity, mortality, and aetiologies of lower respiratory infections in 195 countries, 1990–2016: a systematic analysis for the global burden of disease study 2016. Lancet Infect Dis. 2018;18(11):1191–210.
3. Global mortality associated. With 33 bacterial pathogens in 2019: a systematic analysis for the global burden of disease study 2019. Lancet. 2022;400(10369):2221–48.
4. Campanero-Rhodes MA, Lacoma A, Prat C, García E, Solís D. Development and evaluation of a microarray platform for detection of serum antibodies against Streptococcus pneumoniae capsular polysaccharides. Anal Chem. 2020;92(11):7437–43.
5. Chen H, Liu C. Molecular epidemiology of Streptococcus pneumoniae isosslated from children with community-acquired pneumonia under 5 years in Chengdu, China. Epidemiol Infect. 2022;151:e2.
6. Li L, Ma J, Yu Z, Li M, Zhang W, Sun H. Epidemiological characteristics and antibiotic resistance mechanisms of Streptococcus pneumoniae: an updated review. Microbiol Res. 2023;266:127221.
7. Cools F, Delputte P, Cos P. The search for novel treatment strategies for Streptococcus pneumoniae infections. FEMS Microbiol Rev. 2021;45(4):fuaa072.
8. Scelfo C, Menzella F, Fontana M, Ghidoni G, Galeone C, Facciolongo NC. Pneumonia and invasive Pneumococcal diseases: the role of Pneumococcal conjugate vaccine in the era of multi-drug resistance. Vaccines. 2021;9(5):420.
9. Chien YC, Lee YL, Liu PY, Lu MC, Shao PL, Lu PL, Cheng SH, Lin CY, Wu TS, Yen MY, et al. National surveillance of antimicrobial susceptibilities to Dalbavancin, Telavancin, Tedizolid, Eravacycline, Omadacycline and other comparator antibiotics and serotype distribution of invasive Streptococcus pneumoniae isolates in adults: results from the surveillance of multicenter antimicrobial resistance in Taiwan (SMART) programme in 2017–2020. J Glob Antimicrob Resist. 2021;26:308–16.
10. Li X, Chen Y, Xu X, Li Y, Fan Y, Liu X, Bian X, Wu H, Zhao X, Feng M, et al. Pharmacokinetics and pharmacodynamics of Nemonoxacin in a neutropenic murine lung infection model against Streptococcus Pneumoniae. Front Pharmacol. 2021;12:658558.
11. Wang S, Cai C, Shen Y, Sun C, Shi Q, Wu N, Zheng S, Qian J, Zhang R, Zhou H. In vitro activity of contezolid against Methicillin-Resistant Staphylococcus aureus, Vancomycin-Resistant Enterococcus, and strains with linezolid resistance genes from China. Front Microbiol. 2021;12:729900.
12. Yan Z, Cui Y, Huang X, Lei S, Zhou W, Tong W, Chen W, Shen M, Wu K, Jiang Y. Molecular characterization based on Whole-Genome sequencing of Streptococcus pneumoniae in children living in Southwest China during 2017–2019. Front Cell Infect Microbiol. 2021;11:726740.
13. Page AJ, Cummins CA, Hunt M, Wong VK, Reuter S, Holden MT, Fookes M, Falush D, Keane JA, Parkhill J. Roary: rapid large-scale prokaryote pan genome analysis. Bioinformatics. 2015;31(22):3691–3.
14. Seemann T. Prokka: rapid prokaryotic genome annotation. Bioinformatics. 2014;30(14):2068–9.
15. Lewis I, James S. Performance standards for antimicrobial susceptibility testing. *(No Title)*.
16. Link-Gelles R, Thomas A, Lynfield R, Petit S, Schaffner W, Harrison L, Farley MM, Aragon D, Nicols M, Kirley PD, et al. Geographic and Temporal trends in antimicrobial nonsusceptibility in Streptococcus pneumoniae in the post-vaccine era in the united States. J Infect Dis. 2013;208(8):1266–73.
17. DeAntonio R, Yarzabal JP, Cruz JP, Schmidt JE, Kleijnen J. Epidemiology of community-acquired pneumonia and implications for vaccination of children living in developing and newly industrialized countries: A systematic literature review. Hum Vaccin Immunother. 2016;12(9):2422–40.
18. Huang H, Lin CY, Chiu NC, Huang DT, Huang CY, Chi H. Antimicrobial susceptibility and serotype replacement of Streptococcus pneumoniae in children before and after PCV13 introduction in Taiwan. J Microbiol Immunol Infect. 2023;56(2):299–310.
19. Deurenberg RH, Bathoorn E, Chlebowicz MA, Couto N, Ferdous M, García-Cobos S, Kooistra-Smid AM, Raangs EC, Rosema S, Veloo AC, et al. Application of next generation sequencing in clinical microbiology and infection prevention. J Biotechnol. 2017;243:16–24.
20. Narciso AR, Dookie R, Nannapaneni P, Normark S, Henriques-Normark B. Streptococcus pneumoniae epidemiology, pathogenesis and control. Nat Rev Microbiol 2024.

21. Mendes RE, Hogan PA, Streit JM, Jones RN, Flamm RK. Zyvox® annual appraisal of potency and spectrum (ZAAPS) program: report of linezolid activity over 9 years (2004-12). J Antimicrob Chemother. 2014;69(6):1582–8.
22. Heidary M, Dashtbin S, Asadi A, Asadollahi P, Khatib A, Ebrahimi MA, Ghanbari Z, Darbandi A, Ghanavati R, Pakzad R. Prevalence of linezolid resistance in Streptococcus pneumoniae isolates: a systematic review and meta-analysis. Future Microbiol. 2024;19:449–59.
23. Tsiodras S, Gold HS, Sakoulas G, Eliopoulos GM, Wennersten C, Venkataraman L, Moellering RC, Ferraro MJ. Linezolid resistance in a clinical isolate of Staphylococcus aureus. Lancet. 2001;358(9277):207–8.
24. Prystowsky J, Siddiqui F, Chosay J, Shinabarger DL, Millichap J, Peterson LR, Noskin GA. Resistance to linezolid: characterization of mutations in rRNA and comparison of their occurrences in vancomycin-resistant enterococci. Antimicrob Agents Chemother. 2001;45(7):2154–6.
25. Long KS, Poehlsgaard J, Kehrenberg C, Schwarz S, Vester B. The Cfr rRNA methyltransferase confers resistance to phenicols, Lincosamides, Oxazolidinones, pleuromutilins, and Streptogramin A antibiotics. Antimicrob Agents Chemother. 2006;50(7):2500–5.
26. Wang Y, Lv Y, Cai J, Schwarz S, Cui L, Hu Z, Zhang R, Li J, Zhao Q, He T, et al. A novel gene, OptrA, that confers transferable resistance to Oxazolidinones and phenicols and its presence in Enterococcus faecalis and Enterococcus faecium of human and animal origin. J Antimicrob Chemother. 2015;70(8):2182–90.
27. Bauer G, Berens C, Projan SJ, Hillen W. Comparison of Tetracycline and Tigecycline binding to ribosomes mapped by dimethylsulphate and drug-directed Fe2 + cleavage of 16S rRNA. J Antimicrob Chemother. 2004;53(4):592–9.
28. Veeraraghavan B, Poojary A, Shankar C, Bari AK, Kukreja S, Thukkaram B, Neethimohan RG, Bakhtavachalam YD, Kamat S. In-vitro activity of Tigecycline and comparator agents against common pathogens: Indian experience. J Infect Dev Ctries. 2019;13(3):245–50.
29. Yang Q, Xu YC, Kiratisin P, Dowzicky MJ. Antimicrobial activity among gram-positive and gram-negative organisms collected from the Asia-Pacific region as part of the Tigecycline evaluation and surveillance trial: comparison of 2015 results with previous years. Diagn Microbiol Infect Dis. 2017;89(4):314–23.
30. Gordon NC, Wareham DW. A review of clinical and Microbiological outcomes following treatment of infections involving multidrug-resistant Acinetobacter baumannii with Tigecycline. J Antimicrob Chemother. 2009;63(4):775–80.
31. Lupien A, Billal DS, Fani F, Soualhine H, Zhanel GG, Leprohon P, Ouellette M. Genomic characterization of Ciprofloxacin resistance in a laboratory-derived mutant and a clinical isolate of Streptococcus pneumoniae. Antimicrob Agents Chemother. 2013;57(10):4911–9.
32. Lupien A, Gingras H, Leprohon P, Ouellette M. Induced Tigecycline resistance in Streptococcus pneumoniae mutants reveals mutations in ribosomal proteins and rRNA. J Antimicrob Chemother. 2015;70(11):2973–80.
33. Schmitz J, van der Linden M, Al-Lahham A, Levina N, Pletz MW, Imöhl M. Fluoroquinolone resistance in Streptococcus pneumoniae isolates in Germany from 2004–2005 to 2014–2015. Int J Med Microbiol. 2017;307(4–5):216–22.
34. Pletz MW, Fugit RV, McGee L, Glasheen JJ, Keller DL, Welte T, Klugman KP. Fluoroquinolone-resistant Streptococcus pneumoniae. Emerg Infect Dis. 2006;12(9):1462–3.

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