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Dynamics of serotype 14 *Streptococcus pneumoniae* population causing acute respiratory infections among children in China (1997–2012)

Mingming He[†], Kaihu Yao[†], Wei Shi[†], Wei Gao, Lin Yuan, Sangjie Yu and Yonghong Yang^{*}

Abstract

Background: In the last decade, the *Streptococcus pneumoniae* population has changed, mainly due to the abuse of antibiotics. The aim of this study was to determine the genetic structure of 144 *S. pneumoniae* serotype 14 isolates collected from children with acute respiratory infections during 1997–2012 in China.

Methods: All isolated pneumococci were tested for their sensitivity to 11 kinds of antibiotics with the E-test method or disc diffusion. The macrolides resistance genes *ermB* and *mefA*, as well as the sulfamethoxazole-trimethoprim resistance gene dihydrofolate reductase (DHFR) were detected by polymerase chain reaction (PCR). The sequence types (STs) were analyzed with multilocus sequence typing (MLST).

Results: From 1997 to 2012, the percentage of serotype 14 *S. pneumoniae* isolates in the whole isolates increased. All of the 144 serotype 14 *S. pneumoniae* isolates were susceptible to amoxicillin-clavulanic acid, vancomycin and levofloxacin. No penicillin resistant isolate was found, and the intermediate rate was as low as 0.7 %. Erythromycin resistance was confirmed among 143 isolates. The *ermB* gene was determined in all erythromycin resistant isolates, and the *mefA* gene was positive additionally in 13 of them. The non-susceptibility rate to the tested cephalosporins increased from 1997–2012. All trimethoprim-resistant isolates contained the Ile100-Leu mutation. Overall, 30 STs were identified, among which ST876 was the most prevalent, followed by ST875. During the study period, the percentage of CC876 increased from 0 % in 1997–2000 to 96.4 % in 2010–2012, whereas CC875 decreased from 84.2 to 0 %. CC876 showed higher non-susceptibility rates to β -lactam antibiotics than CC875.

Conclusion: The percentage of serotype 14 *S. pneumoniae* isolates increased over time in China. The increase of resistance to β -lactam antibiotics in this serotype isolates was associated with the spread of CC876.

Keywords: *Streptococcus pneumoniae*, Serotypes, Antibiotic resistance, Children, Epidemiology

Background

Streptococcus pneumoniae (*S. pneumoniae*) is a leading cause of bacterial infections in infants and young children. World Health Organization (WHO) estimated that 1.6 million people die of pneumococcal diseases annually, including 0.7–1 million children aged <5 years mostly from developing countries [1]. Management of *S. pneumoniae* infections has been complicated by the emergence of multiple antimicrobial drug-resistant strains [2].

More than 92 serotypes have been identified based on the discrepancy of capsular polysaccharide antigens. Before the introduction of the pneumococcal conjugate vaccines, serotype 14 *S. pneumoniae* was one of the most common types worldwide [3, 4] and it was usually one common type of antibiotic resistant isolates [5]. Penicillin-resistant and multidrug-resistant pneumococci were known to be restricted to serogroups 23, 6, 19 and serotype 14, which were particularly associated with the infectious diseases in children [6–8]. Spain^{14–5}(ST18), England^{14–9}(ST9), CSR^{14–10}(ST20), Tennessee^{14–18}(ST67), Denmark^{14–32}(ST230) and Netherlands^{14–35}(ST124) were internationally disseminated antimicrobial resistant clones described by the Pneumococcal Molecular Epidemiology

* Correspondence: shiwei613115@126.com

[†]Equal contributors

Key Laboratory of Major Diseases in Children and National Key Discipline of Pediatrics, Ministry of Education, Beijing Pediatric Research Institute, Beijing Children's Hospital Affiliated to Capital Medical University, Beijing, P. R. China

Network (PMEN) (<http://www.sph.emory.edu/PMEN/index.html>).

We reported previously some international spread drug resistant clones in serotype 19 F and 23 F which had been identified in China with increasing frequency [9, 10]. The current study is conducted to analyze the antimicrobial resistance and population biology of serotype 14 *S. pneumoniae* isolates in China.

Methods

Pneumococcal isolates

From 1997 to 2012, a total of 1984 *S. pneumoniae* isolates were collected from children <5 years old with the diagnosis of acute upper respiratory infection in Beijing, Shanghai, Guangzhou, Shenzhen, Chongqing, Xi'an of China. The patients suffering from "lower respiratory tract infections" like pneumonia were excluded because we can not ensure the isolates were from lower respiratory tract or from the nasopharynx. The details of these *S. pneumoniae* collections have been published previously [11–16]. A total of 144 isolates were identified as serotype 14 based on quellung reaction, which was performed using the Pneumotest Kit (Statens Serum Institut, Copenhagen, Denmark). The isolates were cultured from nasopharyngeal specimens, except those collected from Beijing in 2006–2008 and from Shenzhen in 2009–2011, which were cultured from hypopharyngeal aspirate specimens.

All isolates were taken as part of standard patient care. A parent and/or legal guardian of each participant signed a written informed consent document before enrollment and before any study procedure was performed. This study was viewed and approved by the Ethics Committee of Beijing Children's Hospital Affiliated to Capital Medical University. No ethical problems existed in this study.

Antimicrobial susceptibility

The minimum inhibitory concentrations (MICs) of all isolates were determined for penicillin, amoxicillin–clavulanic acid, ceftriaxone, cefuroxime, erythromycin, imipenem, levofloxacin, and vancomycin using E-test strips (AB Biodisk, Solna, Sweden) [17], and their susceptibility to tetracycline, sulfamethoxazole–trimethoprim, and chloramphenicol was assessed with disc diffusion tests (Oxoid). The breakpoints were adopted in accordance with the Clinical and Laboratory Standards Institute 2012 criteria [18]. *Streptococcus pneumoniae* ATCC49619 was used as the reference strain in the susceptibility tests. Isolates were considered multi-drug resistant if they were not susceptible to three or more classes of antimicrobials.

Macrolide-resistance genes

Chromosomal DNA was extracted from overnight cultures of *S. pneumoniae* isolates grown on 5 % trypticase soy agar (Oxoid Ltd, Basingstoke, England) using the SiMax™ Genomic DNA Extraction Kit (SBS Genetech Co., Ltd), according to the manufacturer's instructions. The macrolide-resistance genes *ermB* and *mefA* were detected by polymerase chain reaction (PCR) for all of the erythromycin non-susceptible strains, the primers and PCR procedures were previously described [19].

Multilocus sequence typing (MLST)

All strains were characterized with multilocus sequence typing (MLST). Bacterial chromosomal DNA was extracted as described above. The PCR fragments of the seven MLST genes (*aroE*, *gdh*, *gki*, *recP*, *spi*, *xpt*, and *ddl*) were amplified from the chromosomal DNA. The products were sent to BGI Company (Beijing, China) for sequencing on both strands. The STs were determined by comparing the allelic profiles with the recognized STs at the MLST website (<http://spneumoniae.mlst.net>). The new alleles and allelic profiles identified in the present study have been submitted to the MLST database for name assignment. The eBURST v3 software (available at <http://www.mlst.net>) was used to estimate the relationships among the isolates and to assign

Table 1 Susceptibility and MICs of 144 pneumococcal isolates to 11 antibiotics

Antibiotics	Susceptibility		MIC(μ g/ml)		
	I(%)	R(%)	50 %	90 %	Range
PEN	0.7	0	0.5	1	0.004–3
AMC	0	0	0.38	1	0.008–2
CXM	43.8	7.6	1.5	3	0.008–6
CRO	6.9	1.4	0.38	1	0.004–8
IPM	49.3	0	0.094	0.19	0.004–0.38
ERY	0	99.3	>256	>256	0.125–> 256
LVX	0	0	0.5	0.75	0.25–1.5
VAN	0	0	0.5	0.75	0.094–1
TCY	28.5	56.9	-	-	-
SXT	0	47.2	-	-	-
CHL	0	15.3	-	-	-

PEN penicillin, AMC amoxicillin–clavulanic acid, CXM cefuroxime, CRO ceftriaxone, IPM imipenem, ERY erythromycin, LVX levofloxacin, VAN vancomycin, TCY tetracycline, CHL chloramphenicol, SXT trimethoprim–sulfamethoxazole, - no data for disk diffusion test, I intermediate, R resistant, MIC50 minimum inhibitory concentration at which 50 % of the strains were inhibited, MIC90 minimum inhibitory concentration at which 90 % of the strains were inhibited, MIC range range of minimum inhibitory concentration

strains to a clonal complex (CC) using the stringent group definition of six of seven shared alleles.

Dihydrofolate reductase genes

The dihydrofolate reductase (DHFR) genes were amplified for all isolates with primers previously reported [20] and then sequenced. The sequences were analyzed and compared to each other by MEGA4.1 software.

Statistical analysis

The antibiotic susceptibility data were analyzed using WHONET 5.6 software as recommended by the WHO. The χ^2 test, performed with the SPSS software v. 13.0 (SPSS Inc. USA), was used for statistical comparisons. For effective comparison, the study period was divided into five stages: 1997–2000, 2001–2003, 2004–2006,

2007–2009 and 2010–2012. A two-tailed cut-off of $P < 0.05$ indicated statistical significance.

Results

Frequency of serotype 14 *S. pneumonia* isolates over time

During the study period, serotype 14 isolates was identified in 7.3 % (144/1984) of all of the *S. pneumonia* strains. The frequencies in different stages were 3.2 % (19/593) in 1997–2000, 5.5 % (38/691) in 2001–2003, 7.1 % (24/338) in 2004–2006, 22 % (35/159) in 2007–2009, 13.8 % (28/203) in 2010–2012 ($\chi^2 = 82.069$, $P < 0.05$).

Antibiotic susceptibility

The susceptibility and the MICs of the isolates against 11 antibiotics were presented in Table 1. All of the 144

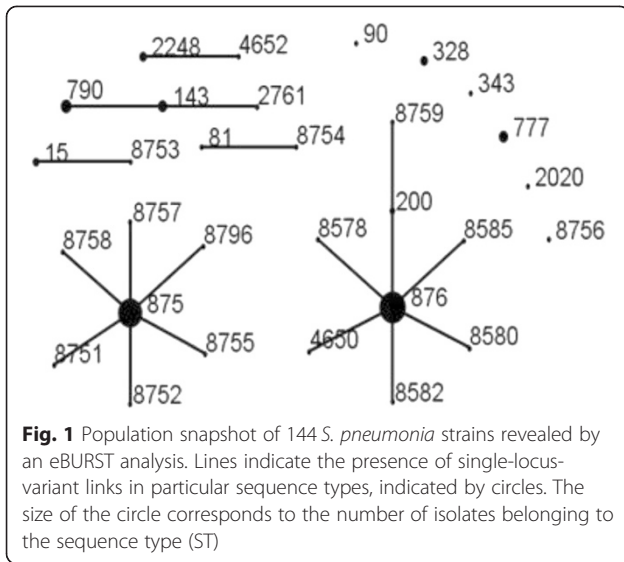
Table 2 Susceptibility of the 144 serotype 14 isolates to eight antimicrobials throughout the study period

Antibiotics	Susceptibility and MIC	1997–2000 (n = 19)	2001–2003 (n = 38)	2004–2006 (n = 24)	2007–2009 (n = 35)	2010–2012 (n = 28)	Total (n = 144)
PEN	I%	0	0	0	2.9	0	0.7
	MIC50($\mu\text{g/ml}$)	0.016	0.032	0.5	0.75	0.5	0.5
	MIC90($\mu\text{g/ml}$)	0.75	0.5	1	1.5	1	1
	MIC Range($\mu\text{g/ml}$)	0.004–1.5	0.008–0.75	0.012–1.5	0.008–3	0.25–1.5	0.004–3
AMC	MIC50($\mu\text{g/ml}$)	0.016	0.023	0.5	0.5	0.5	0.38
	MIC90($\mu\text{g/ml}$)	0.75	0.5	1	1	0.75	1
	MIC Range($\mu\text{g/ml}$)	0.008–1	0.008–1	0.016–2	0.008–1	0.25–1.5	0.008–2
CXM	I%	10.5	13.2	50.0	65.7	75.0	43.8
	R%	0	0	4.2	20.0	10.7	7.6
	MIC50($\mu\text{g/ml}$)	0.023	0.032	1.5	2	2	1.5
	MIC90($\mu\text{g/ml}$)	2	1.5	3	4	4	3
	MIC Range($\mu\text{g/ml}$)	0.016–2	0.008–2	0.016–4	0.032–4	0.25–6	0.008–6
CRO	I%	0	0	4.2	17.1	10.7	6.9
	R%	0	0	0	0	7.1	1.4
	MIC50($\mu\text{g/ml}$)	0.016	0.032	0.5	0.75	0.5	0.38
	MIC90($\mu\text{g/ml}$)	0.38	0.38	1	1.5	2	1
	MIC Range($\mu\text{g/ml}$)	0.004–0.5	0.008–0.75	0.016–1.5	0.008–2	0.25–8	0.004–8
IPM	I%	10.5	18.4	50.0	62.9	100	49.3
	MIC50($\mu\text{g/ml}$)	0.012	0.032	0.094	0.125	0.19	0.094
	MIC90($\mu\text{g/ml}$)	0.125	0.125	0.19	0.19	0.25	0.19
	MIC Range($\mu\text{g/ml}$)	0.004–0.125	0.004–0.19	0.012–0.19	0.012–0.19	0.125–0.38	0.004–0.38
TCY	I%	0	5.3	8.3	40.0	82.1	28.5
	R%	94.7	86.8	75.0	28.6	10.7	56.9
SXT	I%	0	0	0	0	0	0
	R%	84.2	86.8	45.8	20.0	3.6	47.2
CHL	R%	52.6	18.4	20.8	0	0	15.3

PEN penicillin, AMC amoxicillin–clavulanic acid, CXM cefuroxime, CRO ceftriaxone, IPM imipenem, TCY tetracycline, CHL chloramphenicol, SXT trimethoprim–sulfamethoxazole, - no data for disk diffusion test, I intermediate, R resistant, MIC50 minimum inhibitory concentration at which 50 % of the strains were inhibited, MIC90 minimum inhibitory concentration at which 90 % of the strains were inhibited, MIC range range of minimum inhibitory concentration

Table 3 The amino acid mutation of dihydrofolate reductase of trimethoprim-sulfamethoxazole-susceptible and -resistant isolates

Reference sequence ^a	Amino acid	Sequence types of SXT-susceptible isolates																				
		S1 (6)	S2 (15)	S3 (55)	R1 (1)	R2 (1)	R3 (1)	R4 (1)	R5 (1)	R6 (1)	R7 (1)	R8 (1)	R9 (2)	R10 (2)	R11 (3)	R12 (4)	R13 (5)	R14 (6)	R15 (11)	R16 (12)	R17 (15)	
1	Met	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	Ile	-	-
6	Val	-	-	-	-	-	-	-	-	-	-	Ile	-	-	-	-	-	-	-	Ile	-	-
13	Glu	-	-	Lys	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
14	Glu	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	Asp	-
20	Glu	-	-	-	-	Asp	Asp	Asp	Asp	Asp	-	Asp	Asp	Asp	Asp	Asp	Asp	Asp	Asp	Asp	Asp	Asp
26	His	-	-	-	-	Tyr	-	-	Tyr	-	-	-	-	-	Tyr	Tyr	-	Tyr	-	Tyr	-	Tyr
32	Gln	-	-	-	-	-	-	-	-	Lys	-	-	-	-	-	-	-	-	-	Arg	-	-
53	Met	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	Ile	-	-	-
60	Lys	-	-	-	-	-	-	-	-	Gly	-	Gln	-	-	-	-	-	-	-	-	-	-
70	Pro	-	-	-	-	Leu	Ser	-	-	Val	Ser	Leu	Leu	Ser	Ser	Ser	Ser	Ser	Ser	Ser	Ser	Arg
73	Lys	-	-	-	-	-	-	-	-	Ser	-	-	-	-	-	-	-	-	-	-	-	-
74	Ile	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	Leu	-
75	Asp	-	-	-	-	-	-	-	-	Glu	-	-	-	-	-	-	-	-	-	-	-	-
78	Ala	-	-	-	-	Thr	Thr	Thr	Thr	Leu	-	-	-	-	-	Thr	-	-	-	Thr	-	Thr
79	Thr	-	-	-	-	-	-	-	-	Val	-	-	-	-	-	-	-	-	-	-	-	-
81	Gln	-	-	-	-	-	Tyr	Tyr	Tyr	-	Tyr	His	His	Tyr	His	Tyr	His	His	Tyr	His	Tyr	Tyr
82	Asp	-	-	-	-	-	-	-	-	Asn	-	-	-	-	-	-	-	-	-	-	-	-
83	Val	-	-	-	-	Ile	-	-	-	-	-	-	-	-	Ile	-	Ile	-	-	-	-	-
84	Gln	-	-	-	-	-	-	-	-	Glu	-	-	-	-	-	-	-	-	-	-	-	-
85	Ser	-	-	-	-	-	-	-	-	Asp	-	-	-	-	-	-	-	-	-	-	-	-
88	Asp	-	-	-	-	-	-	Gly	-	-	-	-	-	-	-	-	-	-	-	-	-	-
91	Gln	-	-	-	-	-	-	-	-	His	-	-	-	-	-	His	-	-	-	-	His	-
92	Asp	Ala	Ala	Ala	-	Gly	Ala	Ala	Ala	Asn	Ala	Ala	Ala	Ala	Ala	Ala	Ala	Val	Ala	Ala	Ala	Ala
94	Glu	-	-	-	-	Asp	Asp	-	Asp	Asp	Asp	-	-	Asp	Asp	Asp	Asp	-	Asp	Asp	Asp	Asp
99	Ile	-	-	-	-	-	-	-	-	-	-	-	-	-	Val	-	-	-	-	-	-	-
100	Ile	-	-	-	Leu	Leu	Leu	Leu	Leu	Leu	Leu	Leu	Leu	Leu	Leu	Leu	Leu	Leu	Leu	Leu	Leu	Leu
105	Ile	-	-	-	-	-	-	-	-	-	-	-	-	-	-	Val	-	-	-	-	-	-
108	Ala	-	-	-	-	-	-	-	-	Leu	-	-	-	-	-	-	-	-	-	-	-	-
110	Glu	Gly	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
111	Pro	-	Ala	Ala	-	-	Ser	-	-	-	-	Ser	-	-	-	-	-	Ser	Ser	-	Ser	-
116	Val	-	-	-	-	-	-	-	-	Ile	-	-	-	-	-	-	-	-	-	-	-	-
120	His	-	-	-	-	Gln	-	Gln	-	Gln	-	-	-	-	-	Gln	-	-	Gln	-	Gln	-
124	Arg	-	-	-	-	-	-	-	-	Gln	-	-	-	-	-	-	-	-	-	-	-	Lys
130	Tyr	-	-	-	-	-	-	-	-	Phe	-	-	-	-	-	-	-	-	-	-	-	-
133	Glu	-	-	Gly	Lys	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
134	Glu	-	-	-	-	-	-	-	-	Asp	-	-	-	-	-	-	-	-	-	-	-	-
135	Leu	-	-	-	-	-	Phe	Phe	Phe	Phe	Phe	Phe	Phe	Phe	Phe	Phe	Phe	Phe	Phe	Phe	Phe	Phe
137	Leu	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	S	-	-
138	Ser	-	-	-	-	-	-	-	-	Thr	-	-	-	-	-	-	-	-	-	-	-	-
139	Leu	-	-	-	-	Ile	-	-	Ile	Val	Ile	-	-	Ile	-	-	-	-	-	-	-	-
142	Thr	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	Ile	Met	-	-	Pro



Both clones exhibited similar resistance to erythromycin and tetracycline.

Discussion

The present study showed that serotype 14 isolates was not very common in all of the *S. pneumoniae* (7.3 %) strains isolated from Chinese children. But its percentage in the whole *S. pneumoniae* isolates over time increased from 3.2 % in 1997–2000 to 13.8 % in 2010–2012. Epidemiology data in a previous report showed that the percentage of serotype 14 isolates was usually lower than 10.0 % [21]. From January 2005 to December 2006, a total of 451 *S. pneumoniae* isolates were collected from 9 study centers in 8 cities across China, of which

the most prevalent serotypes in descending order were 19 F (42.1 %), 19A (11.8 %), 14 (7.5 %), 6B (7.3 %), 23 F (5.8 %), and 15 (4.4 %) [22]. Our previous study also revealed that among 338 pneumococcal isolates, the most common were 19 F (55.6 %), 19A (13.9 %), 23 F (10.1 %), 6B (4.7 %), and 14 (3.6 %) [23]. But among the isolates causing invasive pneumococcal disease (IPD) in children, serotype 14 may play a more significant role. In a study [14] conducted from 2006 to 2008 in 11 hospitals across China, 171 *S. pneumoniae* isolates collected from children suffering from IPD under the age of 14 were analyzed for serotype distribution, and the most prevalent serotype was 19 F (19.9 %), followed by serotype 14 (19.3 %). In another previous study in China [12], a total of 61 invasive strains were isolated in Shenyang, the serotype distribution of those isolates were 19A (41.0 %), 14 (19.7 %), 19 F (11.5 %) and 23 F (9.8 %). Data from developed countries before the introduction of the conjugated vaccines indicated that the proportion of serotype 14 was 15.7 to 19.8 % [8, 24]. In September 2008, the 7-valent pneumococcal conjugated vaccine (PCV7) became available for the private sector in China. However, the vaccination rate is very low (less than 1.0 %). Thus, the frequency of serotype 14 did not decrease in 2012, and even increased. During the investigation period, the susceptibility to beta-lactam antibiotics of serotype 14 isolates decreased. We also found a significant clonal shift of serotype 14 strains from CC875 to CC876 over time. Given the discrepancy of antibiotic resistance between these two CCs, the genotype replacement was deduced to be caused by the antibiotic selective pressure. The spread of the highly resistant CC876 could be driven by the selective

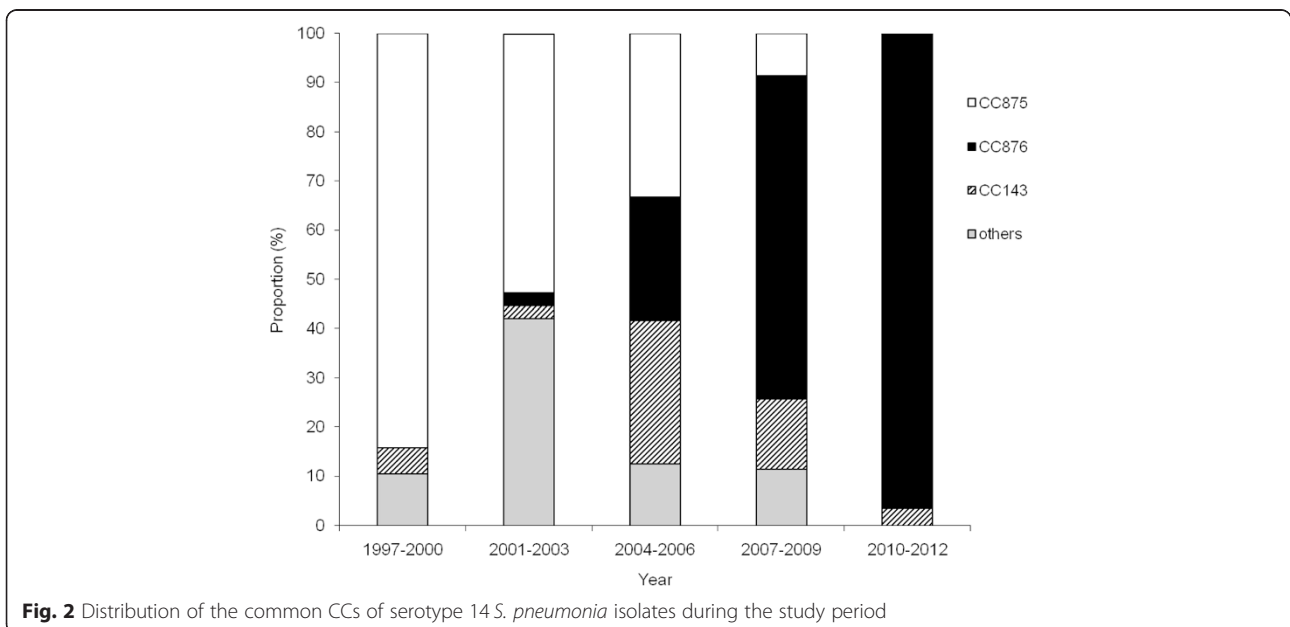


Table 4 Difference between the antimicrobial susceptible profiles of CC876 and CC875 of serotype 14 *S. pneumoniae*

Antibiotics	Susceptibility and MIC	CC876 (n = 57)	CC875 (n = 47)	CC143 (n = 15)	Others (n = 25)	Total
PEN	I%	0	0	6.7	0	0.7
	MIC50(μg/mL)	0.75	0.016	1	0.125	0.5
	MIC90(μg/mL)	1	0.023	1.5	0.75	1
	MIC Range(μg/ml)	0.125–2	0.004–0.032	0.38–3	0.016–1.5	0.004–3
AMC	MIC50(μg/mL)	0.5	0.016	1	0.125	0.38
	MIC90(μg/mL)	1	0.023	1	1	1
	MIC Range(μg/ml)	0.094–1.5	0.008–0.023	0.25–2	0.016–1	0.008–2
CXM	I%	52.6	0	66.7	36	43.8
	R%	36.8	0	26.7	0	7.6
	MIC50(μg/mL)	2	0.023	2	0.5	1.5
	MIC90(μg/mL)	4	0.032	3	2	3
	MIC Range(μg/ml)	0.19–6	0.008–0.125	1–4	0.016–2	0.008–6
CRO	I%	15.8	0	6.7	0	6.9
	R%	3.5	0	0	0	1.4
	MIC50(μg/mL)	0.75	0.023	0.75	0.125	0.38
	MIC90(μg/mL)	1.5	0.032	1	0.5	1
	MIC Range(μg/ml)	0.125–8	0.004–0.047	0.38–1.5	0.016–1	0.004–8
IPM	I%	43.9	0	33.3	4	49.3
	MIC50(μg/mL)	0.125	0.016	0.125	0.064	0.094
	MIC90(μg/mL)	0.19	0.032	0.19	0.125	0.19
	MIC Range(μg/ml)	0.047–0.38	0.004–0.064	0.064–0.19	0.012–0.19	0.004–0.38
TCY	I%	68.4	0	6.7	4	28.5
	R%	5.3	95.7	93.3	80	56.9
SXT	I%	0	0	0	0	0
	R%	3.5	91.5	0	92	47.2
CHL	R%	1.8	31.9	13.3	16	15.3

PEN penicillin, AMC amoxicillin–clavulanic acid, CXM cefuroxime, CRO ceftriaxone, IPM imipenem, TCY tetracycline, CHL chloramphenicol, SXT trimethoprim-sulfamethoxazole, -, no data for disk diffusion test, I intermediate, R resistant, MIC50 minimum inhibitory concentration at which 50 % of the strains were inhibited, MIC90 minimum inhibitory concentration at which 90 % of the strains were inhibited, MIC range range of minimum inhibitory concentration

pressure from antibiotic use which limited the spread of the generally susceptible CC875 at the same time. Previous study suggested that the β -lactam antibiotics, especially the cephalosporin were frequently used in pediatric clinic work [25]. Chloramphenicol has not been used medically since the 1990s, hence, the resistance rate against Chloramphenicol decreased. Our previous study on serotype 19 F [9] and 23 F [10] *S. pneumoniae* isolates indicated that the wide use of beta-lactam antibiotics not only selected the resistant *S. pneumoniae*, but also other epidemiological characteristics, such as serotypes and genotypes. The suggestion is corroborative by the present results.

The genotype replacement of serotype 19 F and 23 F *S. pneumoniae* isolates in China obviously resulted from the input of highly resistant international pneumococcal clones (CC271 and ST81) [9, 10]. Little was known about the spread of serotype 14 *S. pneumoniae*. There

was no sufficient evidence to support that it was associated with some international spread CCs of serotype 14 strains. There were 1594 serotype 14 *S. pneumoniae* isolates in the MLST database (<http://spneumoniae.mlst.net/>) from the whole world. The eBurst analysis of these data showed that the common CCs were as follows: CC15 (32.3 %), CC156 (17.8 % including ST143), CC63 (12.1 %), CC124 (10.7 %) and CC230 (5.7 %). The predominant CC875 and CC876 in the present study covered only 30 (1.9 %) and 13 (0.8 %) strains in the MLST database, respectively. The PMEN notes international spread resistant clones of serotype 14 including ST18, ST9, ST20, ST67, ST230 and ST124, but they were rare in our data.

It was noted that the resistance rate of serotype 14 *S. pneumoniae* isolates to trimethoprim-sulfamethoxazole decreased significantly during the study period. CC876 and CC143 strains were susceptible to trimethoprim-

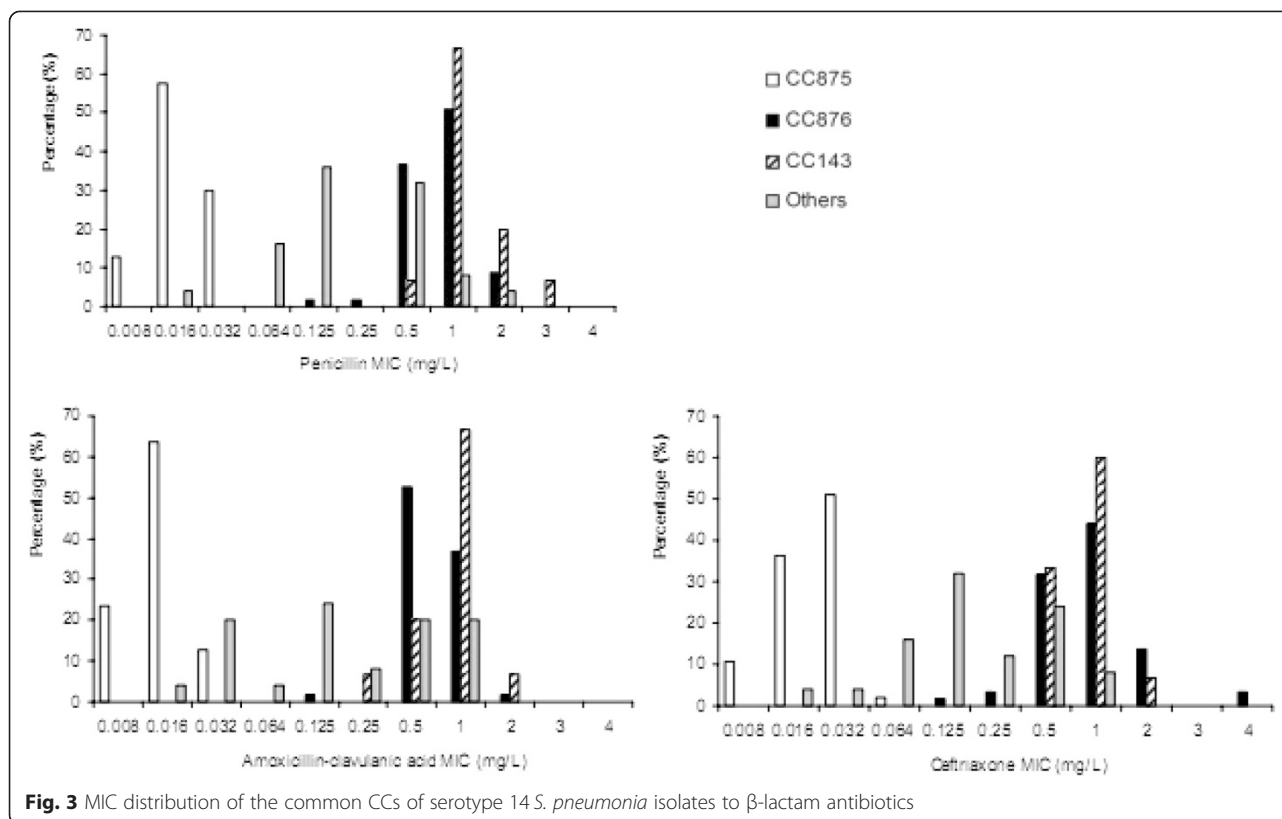


Fig. 3 MIC distribution of the common CCs of serotype 14 *S. pneumoniae* isolates to β -lactam antibiotics

sulfamethoxazole. We did not find the similar results in serotype 19 F [9] and 23 F [10]. The DHFR genes were amplified and sequenced, which confirmed the phenotype test. It was reported [26] that trimethoprim resistance is an essential component of co-trimoxazole resistance. Very few (6 %) of the trimethoprim-resistant isolates were sensitive to co-trimoxazole. A previous study [27] demonstrated that the mutation of Ile100-Leu was critical to the generation of trimethoprim resistance. The results presented here were consistent with the requirement for this mutation but also demonstrated that other changes [28] had considerable impacts on the function of the enzyme. Overall levels of nucleotide divergence from the reference strain were similar in the study, at between 6.0 and 7.5 % for resistant isolates and under 1.5 % for susceptible isolates. The significant epidemiological character should be confirmed by more data.

The study has a limitation. The survey period was divided into five non-equal study period for enough quantity isolates in statistic analysis. Considering the number (144 isolates) of the isolates in this study, we believe that the present result demonstrated the epidemiological changes and molecular characteristics of serotype 14 *S. pneumoniae* in China objectively.

Conclusions

In summary, we found an increase in the prevalence of serotype 14 *S. pneumoniae* isolates with increasing β -lactam antibiotic resistance in China from 1997 to 2012. It was indicated that CC876 replaced CC875 under the pressure of antibiotics. Further long-term surveys of serotype 14 *S. pneumoniae* are required to monitor the prevalent STs and antibiotic resistance of this important human pathogen.

Abbreviations

PCR: Polymerase chain reaction; ST: Sequence type; MLST: Multilocus sequence typing; CC: Clonal complex; MDR: Multidrug resistant; MIC: Minimum inhibitory concentration; DHFR: Dihydrofolate reductase; PCV7: The 7-valent pneumococcal conjugated vaccine; IPD: Invasive pneumococcal disease.

Competing interests

The authors declare that they have no financial or nonfinancial competing interests related to this manuscript.

Authors' contributions

MH, KY, WS, WG, LY, SY, and YY designed the study. WS and SY performed the antimicrobial susceptibility tests. WG and LY performed the molecular epidemiological PCR. MH, KY, WS and YY collected the data, analyzed them, interpreted the results, and drafted the manuscript. All authors reviewed and revised the manuscript and approved the final version.

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