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Original Article

Invasive pneumococcal disease caused by ceftriaxone-resistant *Streptococcus pneumoniae* in Taiwan

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Abstract *Background:* Invasive pneumococcal disease (IPD) was associated with mortality, but the risk factors associated with mortality remains controversial.
Methods: A retrospective cohort study was designed. All patients with IPD from 2011 to 2013 admitted in a medical center were screened and collected for their clinical presentations and laboratory characteristics.
Results: Approximately half of the 134 IPD isolates derived from these patients belonged to three major serotypes (19A, 6A and 3), which are included in 13-valent pneumococcal conjugate vaccine (PCV13), but not in 7-valent pneumococcal conjugate vaccine (PCV7). Ceftriaxone resistance according to non-meningitis criteria was identified in 38% of the IPD isolates, and was the major independent risk factor associated with inappropriate initial therapy that subsequently contributed to mortality of the patients. Infection by serotype 6A, 15B, 19A, 19F, or 23F was the major independent risk factor associated with ceftriaxone resistance (non-meningitis criteria). 77.6% of these isolates belonged to additional PCV13 serotypes, with more than 40% expressing resistance to ceftriaxone. In terms of serotype

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coverage, PCV13 covered 94.1% of the IPD isolates with ceftriaxone resistance, in comparison to 21.6% only by PCV7.
Conclusions: The increase of ceftriaxone resistance in pneumococci in part driven by PCV7 vaccination in Taiwan is worrisome. The use of PCV13 in children as well as in the elderly population is likely to offer protection from the infection caused by ceftriaxone-resistant pneumococci. It is important to give an effective drug such as penicillin, fluoroquinolones or vancomycin in 2 days for improving outcome of IPD patients.

Introduction

Streptococcus pneumoniae is one of the most common pathogens causing community-acquired pneumonia, acute otitis media, and invasive disease such as meningitis and sepsis in humans.[1,2] Invasive pneumococcal disease (IPD) causes 1 million deaths per year worldwide.[3,4] Taiwan has been an epicenter of pneumococcal resistance to penicillin, macrolide, and extended-spectrum cephalosporins as reported in previous studies since 1990s.[5,6] The spread of international clones Taiwan^{19F}-14 and Taiwan^{23F}-15 were documented to cause IPD in many countries in Europe, Africa, and New Zealand.[7–10]

The 7-valent pneumococcal conjugate vaccine (PCV7) was first available in Taiwan in late 2005. A surge of IPD caused by serotype 19A soon occurred, accounting for 53.6% of IPD cases among children aged ≤5 years in 2011–2012.[11] The fact that the prevalence of serotype 19A IPD was first present in children eligible for PCV7 and then extended to other age groups suggested that the serotype replacement caused by PCV7 use contributed to the surge of serotype 19A.[12,13] ST320 has been reported as a major genotype of serotype 19A and was associated with a high drug resistance profile in Taiwan.[14,15] Since ST320 had been increasing even before the introduction of PCV7 in Korea, clonal expansion under antibiotic selective pressure could also play a role for the increase of serotype 19A IPD.[16] The clinical impact of serotype replacement on outcome of the infected patients and how to modify treatment to improve the outcome remain less reported.

This study aimed to apply backward root analysis to survey, step by step, the independent risk factors associated with mortality due to IPD. We analyzed clinical characteristics, microbiological features, and final outcomes of IPD patients stratified by both serotypes and the antimicrobial susceptibility profiles of the isolates.

Materials and methods

Study subjects and inclusion criteria

This study was approved by the Institutional Review Board (103-3935C) of Chang Gung Memorial Hospital (CGMH), Taiwan. Medical records were reviewed for admitted patients who were treated in CGMH from January 2011 to December 2013, had ≥1 positive sterile body site culture for *S. pneumoniae*, and had symptoms and signs of infection. For patients with multiple episodes, only the first episode was included. Patients with incomplete medical records or with polymicrobial infection were excluded.

Clinical data collection and definitions

Clinical data were retrospectively collected. A case of invasive disease was defined when a pneumococcal isolate was recovered from a normally sterile site, including blood, pleural fluid, cerebrospinal fluid, peritoneal fluid, or bone marrow. Additional PCV13 serotypes, mainly serotypes 3, 6 A, and 19A, were defined as serotypes of PCV13 but not PCV7. Sepsis was defined as infection with at least 2 criteria of systemic inflammatory response syndrome (SIRS) met, severe sepsis as sepsis with at least one organ dysfunction, and septic shock as severe sepsis plus one of the shock or hypotension variables.[17] Bacteremic pneumonia/empyema was defined by a clinical diagnosis of pneumonia with a positive culture from blood or pleural fluid.[2] Necrotizing pneumonia was diagnosed if non-enhanced areas appeared on a contrast-enhanced computed tomogram (CT) image or if cavitation (including lung abscess and pneumatoceles) was present on a chest radiograph.[18] Pneumococcal meningitis was defined by the clinical diagnosis of meningitis with *S. pneumoniae* isolated from blood or isolation of *S. pneumoniae* from cerebrospinal fluid.[2] Pneumococcal peritonitis was defined as a clinical diagnosis of peritonitis plus a positive culture from peritoneal fluid or blood.[2] Pneumococcal bone and joint infection was defined as a clinical diagnosis of bone or joint infection with a positive culture from synovial fluid or blood.[2] Primary bacteremia was defined when a positive blood culture was obtained from a patient with fever but without a focus of infection.[2] Sepsis was defined as life-threatening organ dysfunction caused by a dysregulated host response to infection.[19] Mortality was defined as infection-attributable death within 30-day, wherein the criteria of death were met before the symptoms and signs of IPD were resolved and with at least a sterile body site culture positive for *S. pneumoniae*.[20,21] Charlson score, defined as an index of comorbid conditions for patients, counts a total of 22 underlying clinical conditions of patients, such as neoplastic disease, cardiac disease, cerebrovascular disease, diabetes, pulmonary disease, hepatic diseases, renal diseases, or peptic

ulcer.[22] Community-acquired infection (CAI) was defined as the isolation of *S. pneumoniae* from sterile site within 48 h of admission, while hospital-acquired infection (HAI) as beyond that time.[21,23] Appropriate antimicrobial therapy was defined as administering to patients with at least one antimicrobial agent, except aminoglycoside, susceptible in vitro, within 2 days after IPD onset.[20,21] Culture detecting time was the interval (days) from culture sampling to reporting.

Antimicrobial susceptibility

Minimum inhibitory concentrations (MICs) of penicillin and ceftriaxone were analyzed by E-test (AB Biodisk, Solna, Sweden). Susceptibilities to levofloxacin and moxifloxacin were examined by a standard disc diffusion method. According to the guidelines from the Clinical and Laboratory Standards Institute (CLSI), MIC interpretive criteria for penicillin and ceftriaxone are different in meningeal and non-meningeal infections.[24] In the present study, for the consistency of data analysis, antimicrobial susceptibility results were categorized as susceptible (S): penicillin, $\leq$2 μg/mL and ceftriaxone, $\leq$1 μg/mL; and resistant (R): penicillin, >2 μg/mL and ceftriaxone, >1 μg/mL for non-meningeal infection. As for meningeal infection, results were categorized as susceptible (S): susceptible (S): penicillin, $\leq$0.06 μg/mL and ceftriaxone, $\leq$0.5 μg/mL; and resistant (R): penicillin, $\geq$0.12 μg/mL and ceftriaxone, $\geq$1 μg/mL for meningeal infection.[24]

Serotyping

The serotypes of pneumococcal isolates were determined by using antisera (Statens Serum Institut, Copenhagen, Denmark) or PCR methods as previously described.[25–27]

Multi-stages risk factor analysis

An analytical model with multiple stages was proposed from the occurrence of IPD to mortality for patients with mortality.[20,21] The most important main risk factor (MRF1) associated with mortality due to IPD was the first one analyzed by multivariate logistic analysis. Then main risk factor (MRF2) correlated to MRF1 was the second one analyzed by the same method. In this way, MRF1, MRF2, MRF3, MRF4, etc. would be identified as the most important independent risk factor in each stage backward from death to infection.[20,21]

Statistical analysis

Data analyses were performed using SPSS software, v. 17.0 (SPSS Inc., Chicago, IL, USA). The Student's *t*-test, the Chi-square test or Fisher's exact test were used when appropriate to compare proportions. Variables with a *P* value of less than 0.2 in the univariate analysis were added in a forward stepwise manner and selected to create the final model for multivariable analysis. All statistical analyses were two-sided, and significance was set at $P < 0.05$. Quartiles are the breaking values dividing a rank-ordered data into four parts with equal numbers. The breaking values that divide each part are defined as the first, second, and third quartiles, which are denoted by Q1, Q2, and Q3, respectively. Data are presented as median value (interquartile range: Q1–Q3) for continuous variables.

Results

Clinical isolates

A total of 134 clinical IPD isolates, including 116 from blood, 10 from pleural fluid, 6 from cerebral spinal fluid (CSF), 1 from ascites and 1 from synovial fluid in 2011–3 met the inclusion criteria and were collected in this study. According to exclusion criteria, 32 isolates were excluded from this study, including 12 with polymicrobial infection, 18 non-first, repetitive episodes, and 2 insufficient medical records. We have observed no temporal or geographic association among these isolates. There was no outbreak found in the study period.

Serotypes

Serotype distribution from 2011 to 2013 was shown in Table 1. The main serotype from 2011 to 2013 was serotype 19A (32.8%), followed by serotype 14 (12.7%), 6A (9%), 23F (7.5%), 6B (6.7%), and 3 (6%). Serotype 19A was the most predominant serotype in patients aged younger than 50 years old. On the other hand, serotype 6A and 14 were the leading serotype in patients aged 50–64 years and those older than 65 years old, respectively. Monthly distribution of the serotypes was analyzed. IPD occurred more commonly from November to February, the cold season in Taiwan.

Antimicrobial susceptibility

MIC_{50}/MIC_{90} of penicillin and ceftriaxone were 1.5/3 μg/mL and 1/1.5 μg/mL, respectively. Resistance rate to penicillin of more than 50% was found in serotype 6B, 9V, 14, 15A, 15B, 19A, 19F, 23A and 23F according to meningitis criteria; susceptible rate to penicillin of more than 70% was found in all serotypes according to non-meningitis criteria (Table 2). Serotypes 6A, 15B, 19A, 19F and 23F showed more than 40% resistance to ceftriaxone according to both meningitis and non-meningitis criteria. In particular, serotype 19A showed more than 70% resistance to ceftriaxone according to both meningitis and non-meningitis criteria, and 100% resistance to penicillin according to meningitis criteria. All serotypes showed 100% susceptible to vancomycin, levofloxacin and moxifloxacin, except serotypes 14 and 23F, for which 23% and 10%, respectively, showed resistance to levofloxacin and moxifloxacin.

Isolates from all patients showed more than 60% resistance to penicillin according to meningitis criteria but less than 30% resistance according to non-meningitis criteria. Isolates from pediatric patients aged less than 6 years old showed more than 55% resistance to ceftriaxone according to both meningitis and non-meningitis criteria. Isolates from all patients revealed more than 20% resistance to ceftriaxone according to non-meningitis criteria except those from patients aged older than 65 years old (13.3% resistance). Isolates from all age groups showed 100% susceptible to vancomycin, levofloxacin and moxifloxacin, except those from adult patients showing $\leq$10% resistance to levofloxacin and moxifloxacin.

Underlying diseases

Patients aged more than 65 years or infected by serotypes 8, 9V, 11A, and 19F had higher median Charlson index ($\geq$3

Table 1 The percentage of pneumococcal serotypes at different age groups, 2011–2013.

Serotypes	All N = 134	<2 years N = 11	2–5 years N = 36	6–17 years N = 10	18–49 years N = 22	50–65 years N = 25	≥65 years N = 30
PCV7 serotypes	49 (36.6)[a]	5 (45.5)	6 (16.7)	2 (20)	5 (22.7)	10 (40)	20 (66.7)
4	4 (3.0)	0	0	0	1	1 (4)	2 (6.7)
6B	9 (6.7)	1 (9.1)	2 (5.6)	0	0	4 (16)	2 (6.7)
9V	2 (1.5)	0	0	0	1 (4.5)	0	1 (3.3)
14	17 (12.7)	1 (9.1)	2 (5.6)	0	1 (4.5)	3 (12)	10 (33.3)
19F	7 (5.2)	2 (18.2)	1 (2.8)	0	2 (9.1)	1 (4)	1 (3.3)
23F	10 (7.5)	1 (9.1)	1 (2.8)	2 (20)	1 (4.5)	1 (4)	4 (13.3)
Additional PCV13 serotypes	64 (47.8)	6 (54.5)	27 (75.0)	4 (40)	10 (45.5)	11 (44.0)	6 (20.0)
3	8 (6.0)	0	0	2 (20)	3 (13.6)	1 (4)	2 (6.7)
6A	12 (9.0)	0	1 (2.8)	0	3 (13.6)	6 (24)	2 (6.7)
19A	44 (32.8)	6 (54.5)	26 (72.2)	2 (20)	4 (18.2)	4 (16)	2 (6.7)
Non-PCV13 serotypes	21 (15.7)	0 (0)	3 (8.3)	4 (40)	6 (27.3)	4 (16)	4 (13.3)
8	1 (0.8)	0	0	0	1 (4.5)	0	0
11A	1 (0.8)	0	0	0	0	0	1 (3.3)
15A	7 (5.2)	0	1 (2.8)	2 (20)	1 (4.5)	1 (4)	2 (6.7)
15B	4 (3.0)	0	1 (2.8)	1 (10)	0	2 (8)	0
22F	3 (2.2)	0	0	0	1 (4.5)	1 (4)	1 (3.3)
23A	4 (3.0)	0	1 (2.8)	1 (10)	2 (9.1)	0	0
35A	1 (0.8)	0	0	0	1 (4.5)	0	0

[a] Data are presented as number of cases (%) for each categorical variable.

Table 2 Non-susceptibility rates to penicillin and ceftriaxone among different serotypes of *S. pneumoniae*.

Serotypes	N (%)	Penicillin		Ceftriaxone		Levofloxacin	Moxifloxacin	Vancomycin
		Meningitis criteria	Non-meningitis criteria	Meningitis criteria	Non-meningitis criteria			
PCV7 serotypes	49 (36.6)[a]	40 (81.6)	5 (10.2)	31 (63.3)	11 (22.4)	5 (10.2)	5 (10.2)	0
4	4 (3.0)	0	0	0	0	0	0	0
6B	9 (6.7)	7 (87.5)	0	5 (62.5)	0	0	0	0
9V	2 (1.5)	1 (50)	0	1 (50)	0	0	0	0
14	17 (12.7)	15 (88.24)	1 (5.89)	11 (64.71)	2 (11.76)	4 (23.53)	4 (23.53)	0
19F	7 (5.2)	7 (100)	2 (28.6)	4 (57.1)	4 (57.1)	0	0	0
23F	10 (7.5)	10 (100)	2 (20)	10 (100)	5 (50)	1 (10)	1 (10)	0
Additional PCV13 serotypes	64 (47.8)	44 (68.8)	11 (17.2)	44 (68.8)	38 (59.4)	0	0	0
3	8 (6.0)	0	0	0	0	0	0	0
6A	12 (9.0)	11 (91.7)	0	10 (83.3)	5 (41.7)	0	0	0
19A	44 (32.8)	44 (100)	11 (25)	34 (77.3)	33 (75)	0	0	0
Non-PCV13 serotypes	21 (15.7)	14 (66.7)	0	6 (28.6)	2 (9.5)	0	0	0
8	1 (0.8)	0	0	0	0	0	0	0
11A	1 (0.8)	0	0	0	0	0	0	0
15A	7 (5.2)	7 (100)	0	3 (42.9)	0	0	0	0
15B	4 (3.0)	3 (75)	0	3 (75)	2 (50)	0	0	0
22F	3 (2.2)	0	0	0	0	0	0	0
23A	4 (3.0)	4 (100)	0	0	0	0	0	0
35A	1 (0.8)	0	0	0	0 (0)	0	0	0

[a] Data are presented as number of cases (%) for each categorical variable.

points), those aged 19–65 years or infected by serotypes 3, 4, 6A, 6B, 14, 22F and 23A had medium median Charlson index (1–3 points), and those aged ≤18 years or infected by serotypes 15A, 15B, 19A, 23F and 35A showed low median Charlson index (0 point) (Table 3).

The patients aged ≥65 years had the highest median Charlson score 3, followed by those aged 51–65 years (score 2) and those aged 19–50 years (score 1). Patients aged less than 19 years had the lowest median Charlson score 0.

Table 3 Underlying diseases of patients infected by different serotypes of *S. pneumonia*.

Serotypes	N (%)	Age	Charlson index	Neoplasm	Cardiac disease	Stroke	Diabetes	Pulmonary disease	Hepatic disease	Renal disease	Peptic ulcer	Dementia
PCV7 serotypes	49 (36.6)[a]	58.0 (17.1—73.9)	2 (0—3)	16 (32.7)	3 (6.1)	4 (8.2)	10 (20.4)	4 (8.2)	6 (12.2)	8 (16.3)	2 (4.1)	2 (4.1)
4	4 (3.0)	60.4 (47.5—69.5)	2 (1.75—2.5)	1 (25.0)	1 (25.0)	0	2 (50.0)	0	3 (75.0)	0	1 (25.0)	0
6B	9 (6.7)	60.7 (3.5—64.6)	1 (0—6.5)	3 (33.3)	0	1 (11.1)	2 (2.2)	0	1 (11.1)	2 (22.2)	1 (11.1)	0
9V	2 (1.5)	57.4	3	1 (50)	0	0	0	1 (50)	1 (50)	1 (50)	0	0
14	17 (12.7)	68.5 (55—85.9)	2 (0—3)	4 (23.5)	1 (5.9)	3 (17.6)	4 (23.5)	0	1 (5.9)	4 (23.5)	0	2 (11.8)
19F	7 (5.2)	36 (3.3—47.4)	3 (0.5—5)	4 (57.1)	1 (14.3)	0	1 (14.3)	0	0	1 (14.3)	0	0
23F	10 (7.5)	42.3 (6.3—75.7)	0 (0—3.5)	3 (30)	0	0	1 (10)	3 (30)	0	0	0	0
Additional PCV13 serotypes	64 (47.8)	5.5 (3.9—51.1)	2 (0—2)	13 (20.3)	5 (7.8)	3 (4.7)	3 (4.7)	2 (3.1)	2 (3.1)	5 (7.8)	2 (3.1)	1 (1.6)
3	8 (6.0)	41.8 (31.3—58.8)	0.5 (0—2)	2 (25.0)	2 (25.0)	0	0	0	0	1 (12.5)	0	1 (12.5)
6A	12 (9.0)	3.3 (1—54.6)	1.5 (1—3)	7 (58.3)	2 (16.7)	3 (25)	1 (8.3)	1 (8.3)	1 (8.3)	3 (25)	2 (16.7)	0
19A	44 (32.8)	4.1 (3.1—10.9)	0 (0—0)	4 (9.1)	1 (2.3)	0	2 (4.5)	1 (2.3)	1 (2.3)	1 (2.3)	0	0
Non-PCV13 serotypes	21 (15.7)	42.3 (9.4—52.2)	0 (0—2)	0 (0—2)	1 (4.8)	0	5 (23.8)	0	4 (19.1)	2 (9.5)	1 (4.8)	0
8	1 (0.8)	43	8	1 (100)	0	0	1 (100)	0	1 (100)	0	0	0
11A	1 (0.8)	85	5	1 (100)	1 (100)	0	1 (100)	0	0	0	1 (100)	0
15A	7 (5.2)	36 (11.0—61.2)	0 (0—4.5)	3 (42.9)	0	0	2 (28.6)	0	0	0	0	0
15B	4 (3.0)	29.3 (5.9—52.0)	0 (0—1)	0	0	0	1 (25)	0	3 (75)	0	0	0
22F	3 (2.2)	54.4 (46.7—73)	2 (0—2)	2 (66.7)	0	0	0	0	0	0	0	0
23A	4 (3.0)	19.1 (5.5—39.5)	1 (0—2)	0	0	0	0	0	0	2 (50)	0	0
35A	1 (0.8)	34.6	0	0	0	0	0	0	0	0	0	0

[a] Data are presented as median value (interquartile range: Q1—Q3) for continuous variables and number of cases (%) for categorical variables.

Diagnosis and mortality

Bacteremic pneumonia was the most common diagnosis in patients infected by serotype 3, 4, 6B, 14, and 19A but sepsis was the most frequent diagnosis among patients infected by other serotypes (Table 4). Shock has been found in patients infected by serotype 6A, 6B, 14, 15A, 19A, 19F and 22F. Empyema and necrotizing pneumonia have been found in patients infected by serotype 3, 6B, 14, and 19A. Meningitis occurred with a higher incidence ($\geq$30%) in patients infected by serotype 19F, 22F and 23A. Primary bacteremia occurred with a higher incidence ($\geq$30%) in patients infected by serotype 8, 19F, and 22F. Patients infected by 6A and 11A showed a higher mortality ($\geq$50%), while those infected by 3, 14, 15A, 19A, 19F and 23F showed medium mortality (12.5—28.6%).

Patients aged 51—65 years showed the highest mortality (32%), followed by those aged $\geq$65 years (20%), those aged 19—50 years (18.2%), those aged $<$2 years (18.2%), and those aged 2—5 years (5.6%). There was no mortality in patients aged 6—18 years.

Multi-stages risk factor analysis

Many factors correlated with mortality; clinical severity (Pitt bacteraemia score), underlying diseases (Charlson score, neoplastic disease, metastatic malignancy), and inappropriate initial therapy were associated with mortality (Table 5). After multivariate analysis, only infection by serotype 6A, Pitt bacteraemia score, Charlson score, and inappropriate therapy were independent risk factors for 30-day mortality. Among these independent risk factors, inappropriate therapy was the major risk factor (MRF1) for mortality with the highest odds ratio (adjusted odds ratio [AOR], 18.11; $P = 0.001$) (Table 5).

After multivariate analysis, ceftriaxone resistance (non-meningitis criteria) of the isolate was the major independent risk factor (MRF2) associated with inappropriate therapy (AOR, 36.60; 95% confidence interval [CI], 6.70—199.81; $P < 0.001$).

After multivariate analysis, infection by serotypes 6A, 15B, 19A, 19F, or 23F (77.6% [38/49] of these isolates belonged to additional PCV13 serotypes) was the major independent risk factor (MRF3) associated with ceftriaxone resistance (non-meningitis criteria) of the pathogen (AOR, 13.74; 95% confidence interval [CI], 5.64—33.48; $P < 0.001$).

Backward root analysis for mortality

After the above multi-stages risk factor analysis, we were able to summarize stages from infection to mortality due to IPD (Table 6). Infection by serotypes 6A, 15B, 19A, 19F, or 23F (stage 1) was associated with a higher risk of ceftriaxone resistance (non-meningitis criteria) (stage 2). Giving ceftriaxone for empirical treatment of these infections was the most common reason for an inappropriate therapy (stage 3), which was a major risk factor associated with mortality. Early report of ceftriaxone MIC and modifying regimen accordingly in case of resistance are important for reducing mortality.

Discussion

Two important clinical observations are found in this study that enrolled 128 non-meningitis (93.3%) and 6 meningitis cases (6.7%). The non-meningeal isolates resistant to ceftriaxone showed 38% overall, which is the highest ever in the world.[28—31] Broad-spectrum cephalosporins, such as ceftriaxone, are usually recommended to treat IPD hopefully to achieve a better therapeutic outcome.[32—34] The study found that inappropriate initial therapy of giving ceftriaxone to patients infected by ceftriaxone-resistant strains was the major factor correlated to the mortality. On the other hand, additional PCV13 serotypes, including serotype 19A (32.8%), 6A (9%) and 3 (6%), contributed to nearly half of the IPD isolates in our patients, and 96.1% of the isolates with ceftriaxone resistance belonged to PCV13 serotypes, while PCV7 covered only 21.6%. It is apparent that it is better to use PCV13 to prevent IPD caused by resistant strains.

In Taiwan, a catch-up immunization program with PCV13 was launched in 2013 and the national pneumococcal immunization program was implemented in 2015.[35,36] The background behind such vaccination strategy in Taiwan was similar among many Asian countries.[28] Moreover, the predominant serotypes of *S. pneumoniae* were also similar.[28] The conclusion of this study potentially could apply to most Asian countries, where antimicrobial resistance of *S. pneumoniae* is rampant.

Among 785 IPD isolates collected in a medical center in Taiwan from 2000 to 2005, the rate of ceftriaxone non-susceptibility increased from 4.2% in 2000—2004 to 11.5% in 2005 ($P < 0.005$).[6] In another two studies, non-susceptibility rate of ceftriaxone, according to non-meningeal criteria, increased significantly from an average of 2.8% in 2000—05 to 18.4% in 2006—07 ($P < 0.0001$) among 3686 isolates and 13.8% in 81 IPD isolates in 2009—12.[37,38] The average ceftriaxone-nonsusceptible rate among isolates from 11 Asian countries from 2008 to 2009 was 8.6% (ranging from 0% in Japan to 21.2% in Sri Lanka).[28] A review of 12 studies on children with IPD from Europe reported an overall pneumococcal resistance rate to third-generation cephalosporins of 9%.[29] Ceftriaxone non-susceptibility among IPD isolates was $<$10% according to the national laboratory-based pneumococcal surveillance data in South Africa from 2003 to 2010.[27] A total of 8.7% (103/1190) of the *S. pneumoniae* isolates collected in the United States during 2011—12 were ceftriaxone nonsusceptible.[31]

An earlier study from Taiwan showed that four serotypes (6B, 14, 19F, and 23F) accounted for 89.1% (326/366) of the penicillin-nonsusceptible isolates and 90% (27/30) of the ceftriaxone-nonsusceptible isolates.[6] In another study, some of the ceftriaxone-resistant isolates expressed genotypes similar to international multidrug-resistant clones, such as Spain23F, England 14, and Taiwan^{19F}-6 Spain^{23F}-1, Taiwan^{19F}-14, and Taiwan^{23F}-15 were shown to be responsible for the worldwide dissemination of antimicrobial resistance in pneumococci.[2,39] Multidrug resistance was observed in 59.3% of the isolates from Asian countries, and the major serotypes were 19F (23.5%), 23F (10.0%), 19A (8.2%), 14 (7.3%), and 6B (7.3%) (27). We found in this study a positive correlation between the pneumococcal serotypes (6A, 15B, 19A, 19F, and 23F) and the resistance to ceftriaxone, suggesting that in Taiwan an existing selection pressure has been driving the development of ceftriaxone resistance in some clones. Therefore, reducing the

Table 4 Diagnosis and 30-day mortality of patients infected by different serotypes of *S. pneumoniae*.

Serotypes	N (%)	Sepsis	Septic Shock	Bacteremic pneumonia	Empyema	Necrotizing pneumonia	Meningitis	Peritonitis	Bone/joint infection	Primary bacteremia	30-day mortality
PCV7 serotypes	49 (36.6)[a]	35 (71.4)	3 (6.1)	36 (73.5)	6 (12.2)	4 (8.2)	7 (14.3)	1 (2.0)	1 (2.0)	4 (8.2)	7 (14.3)
4	4 (3.0)	3 (75.0)	0	4 (100)	0	0	1 (25)	0	0	0	0
6B	9 (6.7)	6 (66.7)	1 (11.1)	7 (77.8)	3 (33.3)	2 (22.2)	1 (11.1)	0	0	0	0
9V	2 (1.5)	2 (100)	0	2 (100)	0	0	0	0	0	0	0
14	17 (12.7)	13 (76.5)	1 (5.9)	14 (82.4)	3 (17.6)	2 (11.8)	0	0	1 (5.9)	1 (5.9)	3 (17.6)
19F	7 (5.2)	3 (42.9)	1 (14.3)	2 (28.6)	0	0	3 (42.9)	1 (14.3)	0	3 (42.9)	2 (28.6)
23F	10 (7.5)	8 (80)	0	7 (70)	0	0	2 (20)	0	0	0	2 (20)
Additional PCV13 serotypes	64 (47.8)	48 (75.0)	6 (9.4)	53 (82.8)	20 (31.3)	16 (25.0)	6 (9.4)	0	3 (4.7)	4 (6.3)	13 (20.3)
3	8 (6.0)	5 (62.5)	0	6 (75.0)	2 (25)	2 (25)	0	0	0	1 (12.5)	1 (12.5)
6A	12 (9.0)	11 (91.7)	2 (16.7)	10 (83.3)	0	0	2 (18.2)	0	0	1 (8.3)	6 (50)
19A	44 (32.8)	32 (72.7)	4 (9.1)	37 (84.1)	18 (40.9)	14 (31.8)	2 (4.5)	0	0	2 (4.5)	6 (13.6)
Non-PCV13 serotypes	21 (15.7)	16 (76.2)	2 (9.5)	6 (28.6)	0	0	5 (23.8)	2 (9.5)	0	7 (33.3)	2 (9.5)
8	1 (0.8)	0	0	0	0	0	0	0	0	1 (100)	0
11A	1 (0.8)	1 (100)	0	1 (100)	0	0	0	0	0	0	1 (100)
15A	7 (5.2)	5 (71.4)	1 (14.3)	4 (57.1)	0	0	0	0	0	1 (14.3)	1 (14.3)
15B	4 (3.0)	3 (75)	0	0	0	0	0	0	0	3 (75)	0
22F	3 (2.2)	3 (100)	1 (33.3)	1 (33.3)	0	0	1 (33.3)	0	0	1 (33.3)	0
23A	4 (3.0)	3 (75)	0	0	0	0	2 (50)	0	0	1 (25)	0
35A	1 (0.8)	1 (10)	0	0	0	0	1 (10)	0	0	0	0

[a] Data are presented as number of cases (%) for each categorical variable.

Table 5 Logistic regression analysis of risk factors for 30-day mortality.

Characteristics	Non-survivors	Survivors	Univariate analysis[a]	*P*	Multivariate analysis[b]	*P*
	N = 22 n (%)	N = 112 n (%)	Odds ratio (95% CI)		Odds ratio (95% CI)	
Serotypes						
19A	6 (27.3)	38 (33.9)	0.74 (0.27–2.05)	0.562		
14	3 (13.6)	14 (12.5)	1.08 (0.28–4.14)	0.908		
6A	6 (27.3)	6 (5.4)	3.85 (0.99–15.02)	0.052	10.14 (1.07–26.27)	0.044
Demographic characteristics						
Age, years	53.2 (42.2–69.2)	33.0 (4–61.9)	1.02 (1.00–1.04)	0.015		
Sex, male/female	14/8	71/41	1.00 (0.39–2.59)	1.000		
Length of stay in hospital	17 (9–25)	18 (11.3–24.8)	0.94 (0.83–1.06)	0.282		
Underlying diseases						
Charlson score	3 (1.25–5.75)	0 (0–2)	1.39 (1.14–1.62)	0.001	1.72 (1.32–2.24)	< 0.001
Neoplastic disease[c]	13 (59.1)	23 (20.5)	5.78 (2.19–15.24)	<0.001		
Metastatic malignancy[c]	8 (36.4)	9 (8.0)	6.41 (2.13–19.35)	0.001		
Cardiac disease	2 (9.1)	7 (6.2)	1.47 (0.29–7.61)	0.645		
Cerebrovascular disease	3 (13.6)	4 (3.6)	4.18 (0.87–20.20)	0.075		
Diabetes	5 (22.7)	13 (11.6)	2.20 (0.69–6.95)	0.182		
Pulmonary disease	0	6 (5.4)	0.47 (0.02–9.80)	0.625		
Hepatic disease	1 (4.5)	11 (9.8)	0.54 (0.08–3.66)	0.525		
Renal disease	3 (13.6)	12 (10.7)	1.29 (0.33–5.01)	0.714		
Peptic ulcer	2 (9.1)	3 (2.7)	3.57 (0.56–22.72)	0.178		
Clinical severity						
Pitt bacteremia score	5 (3–7)	3 (1–4)	1.59 (1.24–2.03)	<0.001	1.93 (1.38–2.70)	<0.001
White blood cell count, cells/nL	9.90 (4.65–21.58)	13.35 (8.28–21.30)	1.00 (1.00–1.00)	0.370		
C-reactive protein level, mg/L	231.6 (104.1–314.1)	141.8 (53.6–274.5)	1.00 (1.00–1.01)	0.226		
Source						
Blood	21 (95.4)	95 (84.8)	3.76 (0.47–29.82)	0.210		
Pleural fluid	0	10 (8.9)	0.23 (0.01–4.30)	0.322		
Cerebral spinal fluid	1 (4.5)	5 (4.5)	1.02 (0.11–9.17)	0.987		
Diagnosis						
Sepsis	22 (100)	77 (68.8)	20.57 (1.16–236.23)	0.039		
Pneumonia	18 (81.8)	77 (68.8)	2.05 (0.65–6.49)	0.225		
Meningitis	4 (18.2)	14 (12.5)	1.54 (0.46–5.21)	0.488		
Characteristics of infections						
Coinfection with influenza	1 (4.6)	6 (5.4)	0.84 (0.10–7.35)	0.876		
Post-influenza	1 (4.6)	5 (4.5)	1.02 (0.11–9.71)	0.987		
Community-acquired	21 (95.5)	108 (96.4)	1.29 (0.14–12.1)	0.826		
Non-susceptibility (meningitis criteria)						
Penicillin	20 (90.9)	92 (82.1)	2.17 (0.47–10.06)	0.320		
Ceftriaxone	19 (86.4)	72 (64.3)	3.52 (0.98–12.62)	0.054		
Non-susceptibility (non-meningitis criteria)						
Penicillin	4 (18.2)	12 (10.7)	1.85 (0.54–6.39)	0.329		
Ceftriaxone	12 (54.5)	38 (33.9)	2.34 (0.93–5.90)	0.072		
Inappropriate initial therapy	9 (40.9)	9 (8.0)	7.93 (2.67–23.55)	<0.001	18.11 (3.47–94.56)	0.001

[a] Data are presented as median value (interquartile range: Q1–Q3) for continuous variables and number of cases (%) for categorical variables.

[b] All variables with a *P* value < 0.20 in the univariate analysis were considered for inclusion in the logistic regression model in the multivariate analysis. A forward stepwise selection process was utilized. We found that only infection by serotypes 6A, high Charlson score, high Pitt bacteremia score and inappropriate initial therapy were statistically significant risk factors for 30-day mortality.

[c] Neoplastic disease included metastatic malignancy and other neoplastic disease.

Table 6 Backward root analysis for main risk factors at different stages from the development of IPD to mortality and proposed solution for each stage.[a]

Stages	Stage 1	Stage 2	Stage 3	Stage 4
MRF or outcome	Infection by serotypes 6A, 15B, 19A, 19F, or 23F (MRF3)	Infection by strains with ceftriaxone resistance (non-meningitis criteria) (MRF2)	Inappropriate therapy (MRF1)	Mortality due to IPD
Recommended intervention	PCV13 vaccination	Early report of ceftriaxone MIC	Modify regimen if poor response to ceftriaxone	

[a] MRF, main risk factor; IPD, invasive pneumococcal disease; PCV13, 13-valent pneumococcal conjugate vaccine; MIC, minimum inhibitory concentration.

selection pressure by reducing the unnecessary use of extended-spectrum cephalosporins in Taiwan and other Asian countries should be reinforced.

Non-susceptibility to ceftriaxone (non-meningitis criteria) was higher than non-susceptibility to penicillin (non-meningitis criteria) in each age group. Notably, among children aged <2 years, non-susceptibility to ceftriaxone reached 85.7% according to the meningitis criteria, and 72.7% according to the non-meningitis criteria. Resistance to extended-spectrum cephalosporins essentially threatens the successful treatment of IPD. Based on our results, penicillin seemed to have a better in vitro effect than ceftriaxone on non-central nervous system infection caused by *S. pneumoniae*.

In conclusion, the increased use of ceftriaxone to treat IPD pneumococci is worrisome; however, our data revealed that 94.1% of the isolates with ceftriaxone resistance belonged to serotypes covered in PCV13. PCV13 should be used in children as well as in the elderly to reduce infection caused by ceftriaxone-resistant *S. pneumoniae*. Giving an effective drug like penicillin, fluoroquinolones or vancomycin in 2 days is important for improving outcome of IPD patients. The two points are particularly important in areas where the rate of ceftriaxone non-susceptibility of pneumococci is high.

Conflicts of interest

The authors declare that they have no conflicts of interest.

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