1. Study information

Title	Surveillance of pneumococcal serotypes causing invasive pneumococcal disease in children in Japan, 2026-2028: the PneumoCatch 2 study
Protocol number	Pfizer protocol number: 99501715/Mie National Hospital protocol number: 2025-38
Protocol version identifier	V 1.0
Date	17 November 2025
Research question and objectives	Primary objective: To describe the serotype distribution of *Streptococcus pneumoniae* (SP) causing Invasive Pneumococcal Disease (IPD) in Japanese children aged 2 months to less than 5 years during the years 2026-2028. Secondary objectives: 1. To describe the serotype distribution of SP causing IPD in Japanese children aged 5 years to less than 16 years during the years 2026-2028. 2. To determine the antimicrobial susceptibility profiles of pneumococcal isolates causing IPD in Japanese children aged 2 months to less than 16 years during the years 2026-2028. Exploratory objectives: 1. To describe the demographic and clinical characteristics of IPD patients aged 2 months to less than 16 years during the years 2026-2028, including underlying medical conditions (e.g., immunosuppression, chronic heart/lung disease), geographical distribution, and vaccination history. 2. To determine the sequence types (STs) and antimicrobial-resistance genes detection of pneumococcal isolates causing IPD in Japanese children aged 2 months to less than 16 years during the years 2026-2028, using multilocus

	sequence typing (MLST) and whole-genome sequencing (WGS) during the years 2026-2028.
Country of study	*Japan*
Author	Takao Fujisawa

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2. LIST OF ABBREVIATIONS

Abbreviation	Definition
CSF	cerebrospinal fluid
CTX	Cefotaxime
EM	Erythromycin
IPD	Invasive pneumococcal disease
IRB	Institutional Review Board
LFX	Levofloxacin
MDR	Multidrug resistant
MIC	Minimum Inhibitory Concentration
MEM	Meropenem
MLST	Multilocus Sequence Typing
NIID	National Institute of Infectious Disease
NIP	National Immunization Program
NT	Non-typeable
NVST	Non-vaccine serotype (non PCV20 serotype)
PG	Penicillin
PCV	Pneumococcal conjugate vaccine
PPSV	Pneumococcal Polysaccharide Vaccine

SP	*Streptococcus pneumoniae*
ST	Sequence Type
VST	Vaccine serotype

3. RESPONSIBLE PARTIES

Principal Investigator(s) of the Protocol

Name, Degree(s)	Job Title	Affiliation	Address
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Satoshi Nakano, MD, PhD (co-PI)	Senior Researcher	The Japan Institute for Health Security/National Institute of Infectious Diseases (JIHS/NIID)	1-23-1 Toyama, Shinjuku-ku, Tokyo, 162-8640, Japan

External Venders

Vender Name	URL	Role
Mediford corporation	https://www.mediford.com/	・ IPD serotyping using Quellung reaction ・ Antimicrobial susceptibility testing
CTD. Inc.	https://c-ctd.co.jp/	・ Data managed using a secure electronic data capture (EDC) system
JIHS/NIID (Japan Institute for Health Security/National Institute of Infectious Disease)	https://www.jihs.go.jp/index.en.html	・ Multilocus sequence typing ・ Whole-genome sequencing

Keio University Department of Biostatistics	https://biostat.med.keio.ac.jp/	・ Statistical Analysis (Final)

4. ABSTRACT

Title	Surveillance of pneumococcal serotypes causing IPD in children in Japan, 2026-2028, the PneumoCatch 2 study
Rationale and background	Pneumococcal conjugate vaccines (PCVs) have significantly reduced IPD in children, though non-vaccine serotypes have emerged (https://ipd-information.com/child/overview/). In Japan, PCV15 and PCV20 were recently introduced into the National Immunization Program (NIP), and further changes in serotype distribution, antimicrobial resistance, and disease burden are anticipated. However, current national surveillance lacks key clinical, demographic, and vaccination data, highlighting the need for enhanced surveillance to evaluate the real-world impact of these newer PCVs.
Research question and objectives	To monitor serotype distribution and antimicrobial susceptibility of *Streptococcus pneumoniae* causing IPD in Japanese children aged 2 months to less than 16 years after PCV15/PCV20 introduction, through enhanced surveillance
Study Design	This prospective, enhanced surveillance study will collect clinical data and SP isolates from Japanese children aged 2 months to less than 16 years with IPD during the years 2026-2028. Isolates will undergo serotyping (Quellung reaction), antimicrobial susceptibility testing, multilocus sequence typing (MLST), antimicrobial-resistance genes detection, penicillin-binding protein (PBP) typing, and pilus typing. Data will be analyzed to evaluate serotype distribution, resistance patterns, and clonal relationships in relation to clinical characteristics and vaccination history.
Population "Population" includes the setting and study population	The study includes Japanese children aged 2 months to less than 16 years with IPD diagnosed between 2026 and 2028. Cases will be enrolled through "PneumoCatch (http://pneumocatch.jp/)," a nationwide surveillance network of medical institutions capable of submitting isolates and clinical data.
Variables include exposures, outcomes, and key co-variates	**Demographic:** sex, age at onset, prefecture of residence, pneumococcal vaccination history, underlying medical conditions, childcare attendance, siblings and their childcare attendance, household smoking **Clinical characteristics:** clinical diagnosis (bacteremia, meningitis, bacteremic pneumonia, mastoiditis, peritoneal or pleural fluid, and any other isolate collected from a site considered to be other IPD, specimen source (blood, CSF, joint fluid, peritoneal fluid, pericardial fluid and pleural fluid), clinical outcome

	(recovery, sequalae, death). **Key covariables:** sex, age at onset, prefecture of residence, pneumococcal vaccination history, underlying medical conditions.
Data source	Demographic and clinical data, including vaccination history, will be entered into an EDC system by physicians or staff of participating sites based on information obtained from the mother and child health handbook (Boshi-techo) and standard of care caregiver interviews. In Japan, this handbook is a widely used and reliable record of childhood immunizations, typically completed by healthcare providers and maintained by parents. (PCV vaccination history will be confirmed by the mother and child health handbook.) All study data will be submitted through a centralized web-based EDC system managed by the data coordinating center. Participating institutions will include pediatric facilities across Japan that diagnose and treat IPD and are capable of submitting SP isolates along with relevant clinical data.
Study size	Based on previous surveillance (496 cases in Japanese children aged 2 months to less than 16 years, 2015-2017), we estimate 500 IPD cases in children aged 2 months to less than16 years over a period of 3 years. This sample size enables stratified analyses and provides ≥80% power to detect a 10-15% difference in vaccine-type vs. non-vaccine-type proportions (α=0.05).[1]
Data analyses	Serotype-specific isolation rates of SP will be calculated and stratified by age group. Patient characteristics and vaccination history will be summarized descriptively. Temporal trends in vaccine-type and non-vaccine-type isolates will be described. Antimicrobial susceptibility will be summarized according to CLSI criteria, including multidrug resistance. Multilocus Sequence Typing (MLST) will be used to evaluate sequence types (STs) and clonal complexes. Antimicrobial resistance gene detection, including PBP typing and pilus typing, will be conducted using whole-genome sequencing. The data will be analyzed using an in-house prebuilt pipeline at the National Institute for Health Security. Statistical analyses will include Cochran-Armitage trend tests and chi-square tests; p-values <0.05 will be considered significant. Associations between clinical characteristics, comorbidities, outcomes, pneumococcal profiles, and vaccination status will be summarized descriptively in exploratory.

5. AMENDMENTS AND UPDATES

Version Identifier	Date	Amendment Type (substantial or administrative)	Protocol Section(s) Changed	Summary of Amendment(s)	Reason
N/A	N/A	N/A	N/A	N/A	N/A

6. MILESTONES

Milestone	Planned Date
Start of data collection	*01 January 2026*
End of data collection	*31 December 2028*
Study progress report	*30 November 2026*
Interim report 1	*30 November 2027*
Interim report 2	*30 November 2028*
Final report	*30 November 2029*
Publication	*30 January 2030*

7. RATIONALE AND BACKGROUND

SP remains a major cause of bacterial infections in children worldwide[2]. In recent years, pneumococcal conjugate vaccines (PCVs) targeting multiple capsular serotypes have been developed and widely introduced. In the United States, the implementation of PCVs led to a marked decrease in the incidence of IPD in children, demonstrating their substantial preventive impact [3, 4]. However, following the introduction of PCVs, many countries have reported serotype replacement, with an increasing proportion of IPD cases caused by non-vaccine serotypes (NVTs) [1, 5-8].

In Japan, the 7-valent PCV (PCV7) was introduced in 2010 using a 3+1 schedule and became part of the National Immunization Program (NIP) in April 2013. It was replaced by PCV13 in November 2013. Most recently, the 15- and 20- valent PCVs (PCV15 and PCV20, respectively) were introduced in 2024-PCV15 in April and PCV20 in October, with PCV20 becoming the preferred vaccine in the NIP for Japanese children (https://www.mhlw.go.jp/content/10900000/001309951.pdf). Consequently, Japanese children currently have diverse vaccination histories, having received different PCVs depending on the timing of their immunizations.

Similar to the global trends, a significant decrease in vaccine-type IPD and a corresponding increase in NVT IPD have been observed following the introduction of PCVs in Japan (https://ipd-information.com/child/overview/). The Pneumotach (http://PneumoCatch.jp/) has conducted IPD surveillance since 2012, focusing on serotype distribution among clinical isolates and serotype replacement [1][8]. Current national IPD surveillance lacks critical patient-level data, including age, sex, vaccination history, and clinical outcomes. Therefore, enhanced surveillance is essential to monitor

changes in IPD serotype distribution, antimicrobial resistance, and clinical characteristics following the introduction of PCV15 and PCV20.

This study will describe the bacteriological and clinical characteristics of pediatric IPD in Japan from 2026 to 2028, with a focus on serotype evolution, antimicrobial susceptibility, sequence types (STs), and host factors, stratified by Pneumococcal vaccination history (PCV7, 13, 15, 20 or PPSV23).

8. RESEARCH QUESTION AND OBJECTIVES

The overall goal of the study is to describe the evolution of pneumococcal serotypes and clones causing IPD in Japanese children between 2026 and 2028, stratified by age and vaccination status. The study also aims to characterize the role of comorbidities in clinical outcomes and to assess trends in antimicrobial susceptibility and pneumococcal sequence types (STs) over time.

The specific study objectives and endpoints are detailed in Table 1.

Table 1. Primary, secondary, and exploratory objectives and endpoints.

Objectives	**Endpoints**
Primary	**Primary**
To describe the IPD serotype distribution of IPD cases in Japanese children aged 2 months to <5 years during the years 2026-2028.	1. Distribution of serotypes causing IPD for the following age groups: aged 2 to <12 months, 1 to <5 years
Secondary	**Secondary**
1. To describe the serotype distribution of IPD cases in Japanese children aged 5 to <16 years during the years 2026–2028.	1. Distribution of serotypes causing IPD in Japanese children aged 5 to <16 years.
2. To determine the antimicrobial susceptibility of pneumococcal isolates causing IPD in Japanese children aged 2 months to <16 years and to assess trends following PCV20 introduction during the years 2026-2028.	1. The proportion of isolates classified as susceptible and non-susceptible to each tested antibiotic. 2. The proportion of isolates that are susceptible or non-susceptible by serotypes.
Exploratory	**Exploratory**
1. To describe the demographic and clinical characteristics of high-risk children with IPD aged 2 months to <16 years during the years 2026-2028.	1. Proportion of IPD cases with underlying risk conditions, stratified by age group: 2 to <12 months, 12 months to <5 years, 5 to <16 years, and <5 years.
2. To describe the sequence types (STs) of pneumococcal isolates causing IPD in Japanese children aged 2 months to <16 years during the years 2026-2028.	1. Distribution of sequence types and clonal complexes of isolates and their relationship to serotypes and resistance profiles.
3. To describe the prevalence of antimicrobial resistance genes, PBP profile and pilus profile of pneumococcal isolates causing IPD	1. Distribution of antimicrobial resistance genes including PBP profile and pilus type and their relationship to serotypes, ST, and resistance profile.

Objectives	Endpoints
in Japanese children aged 2 months to <16 years during the years 2026-2028.	
4. To describe the serotype distribution of IPD cases in Japanese children based on PCV vaccination history aged 2 months to <16 years during the years 2026–2028	1. Annual number of IPD cases stratified by PCV vaccination history in this age group.

9. RESEARCH METHODS

9.1. Study Design

i. **Enhanced Surveillance**: PneumoCatch (http://pneumocatch.jp/), one of the largest nationwide surveillance networks for IPD in Japan, was established in 2012. Initially funded by Pfizer as the Invasive *Streptococcus pneumoniae* Research (ISR) project, the network conducted national surveillance during two main periods: 2012-2014 and 2015-2017 [1, 8]. The project ended in 2023. As of 2025, both PCV15 and PCV20 are included in the NIP for Japanese children aged 2 months to less than 5 years (three primary doses and one booster dose).
In this new surveillance phase (2026-2028), physicians participating in this study will report the cases of pediatric IPD through a centralized EDC system in updated homepage of PneumoCatch and obtain a unique ID of each patient. Clinical isolates of SP from sterile body sites (e.g., blood, cerebrospinal fluid, or joint fluid) as the standard of care will be submitted to:
Mediford Corporation (https://www.mediford.com/en/) for serotyping (Quellung reaction) and antimicrobial susceptibility testing;
Japan Institute for Health Security/National Institute of Infectious Disease (JIHS/NIID) (https://www.niid.jihs.go.jp/) for Multilocus Sequence Typing (MLST) and antimicrobial resistance gene detection, including PBP typing and pilus typing.
Patient background information-including age, sex, residential prefecture, underlying medical conditions, and PCV vaccination history-will be entered by the participating physicians into the EDC system in the online network in PneumoCatch (http://PneumoCatch.jp/medical/document.html). These data will be collected based on the standard of care medical interviews with parents or caregivers and verified as appropriate using the Mother and Child Health Handbook, a widely adopted and reliable source for immunization records in Japan.

ii. **Case definition:** Study participants will include children aged 2 months to less than 16 years diagnosed with IPD. IPD is defined as the isolation of *Streptococcus pneumoniae* from a normally sterile body site, such as blood, cerebrospinal fluid, or joint fluid. Samples from saliva or respiratory tract samples such as nasopharyngeal swabs (non-sterile sites) will not be included in the study.
In principle, one standard of care isolate per patient per case will be used for study purposes. When

multiple isolates with the same serotype are recovered from the same patient and infection episode*, only one representative isolate will be used for the study. If SP is also isolated from non-sterile sites (e.g., sputum) in addition to a sterile site, the case will still be considered an IPD case however only the isolate from the sterile site will be submitted for serotyping.
For example, a patient with suspected pneumococcal pneumonia from whom SP is isolated from blood will be classified as having IPD, regardless of whether pneumonia is confirmed by chest X-ray. Chest radiography will not be required to meet the IPD case definition [1].
*New episode is defined as diagnosis of IPD at least 30 days after previous diagnosis of IPD.

iii. **Risk factors for IPD [9]**

All information including samples for patients with IPD will be obtained through standard of care with caregiver interviews.

Risk factors for IPD include:

- Cerebrospinal fluid leak
- Chronic heart disease
- Chronic kidney disease (excluding maintenance dialysis and nephrotic syndrome, which are included in immunocompromising conditions)
- Chronic liver disease
- Chronic lung disease (including moderate persistent or severe persistent asthma)
- Cochlear implant
- Diabetes mellitus
- Immunocompromising conditions (on maintenance dialysis or with nephrotic syndrome; congenital or acquired asplenia or splenic dysfunction; congenital or acquired immunodeficiencies
- Diseases and conditions treated with immunosuppressive drugs or radiation therapy, including malignant neoplasms, leukemias, lymphomas, Hodgkin disease, and solid organ transplant
- HIV infection
- Sickle cell disease or other hemoglobinopathies

iv. **Laboratory procedures** (Refer to the implementation plan of the laboratory for details)

SP isolates will be collected as the standard of care and identified by standard procedures at clinical laboratories in participating sites. IPD isolates are stored and shipped for study-specific testing, as described below.

v. **Isolates and serotyping, antimicrobial susceptibility, multilocus sequence typing (MLST), and antimicrobial resistance gene using whole-genome sequencing:**
The presence of *SP* will be confirmed using standard microbiological methods, including optochin sensitivity and bile solubility, in accordance with Clinical and Laboratory Standards Institute (CLSI) guidelines. Serotyping will be performed by Mediford using antisera from the Statens Serum Institute (Copenhagen, Denmark) via the Quellung reaction Serotyping will be conducted for 45 serotypes and then conducted for the additional serotypes if necessary. Serotype testing will continue through all possible serotypes until the serotype is identified.
Antimicrobial susceptibility testing* will be conducted at Mediford Corporation using the broth microdilution method for the following antibiotics: penicillin (PCG), erythromycin (EM), ceftriaxone (CTRX), trimethopri/sulfamethoxazole (TPM/SMX), meropenem (MEM), levofloxacine (LFX), and vancomycin (VAC). The CLSI breakpoints (M-10-ED 35: 2025) will be applied for interpreting minimum inhibitory concentrations (MICs).
Multilocus sequence typing (MLST) and whole-genome sequencing will be performed at [10] following previously established protocols [10]. Sequence types (STs) will be assigned using MLST online database (www.pubmlst.org). Clonal complexes (CCs) will be determined using the goeBURST algorithm implemented in PHYLOViZ (https://www.phyloviz.net/), by grouping sequence types (STs) that share six out of seven loci.
Antimicrobial resistance gene detection including PBP typing and pilus typing will be conducted using whole-genome sequencing. An in-house pneumococcal genome analysis pipeline was developed in a previous surveillance study and will be reused in this study with modifications, including database updates. Associations with known Pneumococcal Molecular Epidemiology Network (PMEN) clones will also be determined where applicable.

* Pfizer antibiotics in Japan will be NOT used as test reagents in antimicrobial susceptibility testing.

9.2. Setting

9.2.1. Inclusion Criteria

This study is designed to analyze SP isolates collected from pediatric cases of IPD. Clinical information will be obtained retrospectively from medical records and not through direct patient enrollment or intervention.

The Inclusion criteria are as follows:

1. The children aged 2 months to less than 16 years at the time of specimen collection between 2026 and 2028.
2. The child is clinically diagnosed with IPD, including but not limited to:
   - Bacterial meningitis
   - Bacteremia
   - Pneumonia (bacteremic pneumonia or empymea)

- Septic arthritis
- Peritonitis, etc.
- Any other patient with SP from sterile fluid

3. SP sample is available for the child and is isolated from a normally sterile site, such as:
   - Cerebrospinal fluid (CSF)
   - Blood
   - Pleural fluid
   - Synovial fluid
   - Pericardial fluid or heart value culture
   - Bone fluid / Biopsy Joint fluid
   - Peritoneal fluid
   - Mastoiditis (bone, tissue, or abscess specimen), or other sterile body fluid

9.2.2. Exclusion Criteria

The following patients will be excluded from the study:

1. Isolates collected from the patient are not confirmed as SP
   - Isolates lacking confirmation by standard microbiological methods (e.g., optochin sensitivity, bile solubility).
2. Isolates collected from the patient are not derived from normally sterile sites
   - For example, isolates obtained solely from nasopharyngeal swabs, sputum, saliva, or other non-sterile sources.
3. Basic clinical information not available for the patient.
   - Patients lacking essential background data (age, diagnosis, specimen source) required for core analysis will not be included.

Patients can be enrolled in the study more than once as long as there are at least 30 days between diagnoses of IPD. A new patient identifier will be assigned for each enrollment.

9.3. Variables

Variable	Description	Data Source(s)	Operational Definition
Age at diagnosis	demographics/patient characteristics	Clinical record	Age in months/years at the time of IPD diagnosis (categorized: 2-11 months, 12-59 months, 5-15 years, Collect age in months)

Sex	demographics/patient characteristics	Clinical record	Male or Female, as recorded by the attending physician
Prefecture of residence	demographics/patient characteristics	Clinical record	The prefecture where the patient resides at the time of diagnosis
Hospital Name	demographics/patient characteristics	Clinical record	Hospital Name
Diagnosis	clinical	Clinical record	Clinical syndrome classified as bacteremia, meningitis, pneumonia, arthritis, bone infection, peritoneal infection, pericardial infection, mastoiditis
Specimen type	Inclusion criterion	Microbiology lab submission form	Type of sterile body fluid (e.g., blood, CSF, pleural fluid) from which *S. pneumoniae* was isolated.
Date of Specimen Collection	clinical	Microbiology lab submission form	Date of collection in Year-Month-Day Format
Underlying medical condition(s) (https://www.cdc.gov/acip/evidence-to-recommendations/pcv20-child-risk-based-etr.html?CDC_AAref_Val=https://www.cdc.gov/vaccines/acip/recs/grade/PCV20-child-risk-based-etr.html#:~:text=cerebrospinal%20fluid%	Risk factor	Clinical record, caregiver interview	Underlying medical conditions including cerebrospinal fluid leak; chronic renal failure or nephrotic syndrome; cochlear implant; congenital or acquired asplenia or splenic dysfunction; congenital or acquired immunodeficiencies; diseases and conditions treated with immunosuppressive drugs or radiation therapy, including malignant neoplasms, leukemias, lymphomas, Hodgkin disease, and solid organ transplant; HIV infection; sickle

20leak,and%20diabetes%20mellitus.)			cell disease and other hemoglobinopathies. For children aged 2-5 years, chronic heart or lung disease, and diabetes mellites will also be included.
IPD Severity	Clinical characteristic	Clinical record	ICU admission (Yes/No), outcome (Alive/Died), sequalae (Yes/No; if Yes, specify), Oxygen use (Yes/No)
Pneumococcal vaccination history	Vaccination history	Mother and Child Health Handbook	Number, date received, and type of PCV (PCV7, 13, 15, 20) or PPSV23 doses* received
Childcare attendance	Risk factor	Caregiver interview via attending physician	Attending daycare/preschool at time of diagnosis (Yes/No)
Sibling status	Risk factor	Caregiver interview via attending physician	Presence and age of siblings, and whether they attend group childcare
Household smoking	Risk factor	Caregiver interview via attending physician	Presence of smokers living in the same household (Yes/No)
Clinical outcome	Clinical	Clinical record	Final outcome: Recovered / Sequelae / Deceased / Ongoing treatment
Serotype of isolate	Primary outcome	Mediford lab	Serotyping for all isolates from participating sites will be conducted by Quellung reaction using antisera of 45 serotypes (1, 2, 3, 4, 5, 6A, 6B, 6C, 6D, 7C, 7F, 8, 9N, 9V, 10A, 11A,

			12F, 13, 14, 15A, 15B, 15C, 15F, 16F, 17F, 18C, 19A, 19F, 20, 21, 22F, 23A, 23B, 23F, 24, 24B, 24F, 28A, 29, 31, 33F, 34, 35B, 37, 38) as the standard testing. For the unidentified serotypes by the standard testing, the additional serotyping will be conducted by Quellung reaction using antisera of different serotypes from initial 45 serotypes.
Antimicrobial susceptibility	Secondary outcome	Mediford lab	Classification (Susceptible / Intermediate / Resistant) by CLSI breakpoints (MIC-based)
Sequence type (ST) / Clonal complex (CC)	Exploratory outcome	JIHS/NIID lab	MLST-derived sequence type and associated clonal complex (via eBURST, PMEN database)
Antimicrobial resistance gene detection	Exploratory outcome	JIHS/NIID lab	Whole-genome sequencing analysis

*Pneumococcal vaccination history is defined as pneumococcal vaccines given at least 14 days prior to the onset of IPD.

9.4. Data Sources

Medical charts of Japanese children aged 2 months to less than 16 years who are diagnosed with IPD at medical institutions participating in the PneumoCatch2 surveillance network during the study period (2026-2028) will be used for retrospective data collection. SP isolates obtained from sterile body sites as standard of care will undergo serotyping, antimicrobial susceptibility testing, and MLST. Patient demographic and clinical data (e.g., age, sex, diagnosis, vaccination history, underlying conditions, outcomes) will be entered into the EDC system by the participating physicians based on medical records using the Mother and Child Health Handbook and the interview with parents conducted as the standard of care in Japan.

9.5. Study Size

Based on previous national surveillance from 2015 to 2017, which identified 496 pediatric IPD cases aged 2 months to less than 16 years, we estimate approximately 500 IPD cases in children aged 2 months to less than 16 years will be collected during the 2026-2028 study period. This sample size will enable stratified analyses by age group, pneumococcal serotype, and vaccination history, and is sufficient to detect a 10-15% difference in vaccine-type vs. non-vaccine-type IPD with ≥80% power at a 0.05 significance level [1].

9.6. Data Management

All case data and laboratory results will be collected and managed using a secure EDC system operated by CTD Inc. Participating sites will enter clinical information-including demographics, diagnosis, vaccination history, and comorbidities-based on medical records and caregiver interviews as the standard of care using the mother and child health handbook. Data entry will be monitored monthly for completeness and accuracy.

Each patient will be assigned a unique study ID that links clinical data with microbiological test results. Serotyping and antimicrobial susceptibility testing results from Mediford Corporation, as well as sequence typing and WGS data from the Japan Institute for Health Security/National Institute of Infectious Disease (JIHS/NIID), will be matched with patient data through the EDC system.

The data management office (CTD Inc.) will oversee data quality control, ensure timely data entry, manage study progress, and facilitate communication among participating sites under the direction of the principal investigator. Personal identifiers will not be included in the analytic dataset to maintain patient confidentiality.

9.6.1. Case Report Forms/Data Collection Tools/Electronic Data Record

Clinical and epidemiological data will be collected using standardized electronic case report forms (eCRFs) integrated into the secure EDC system managed by CTD Inc. Participating sites will complete eCRFs based on medical records and interviews with caregivers. Laboratory data-including serotype, antimicrobial susceptibility, MLST, and WGS results-will be centrally entered or linked via study-specific identifiers. All data entries will be monitored and validated for accuracy and completeness by the data management office.

9.6.2. Record Retention

All study data and associated documentation will be retained in accordance with applicable regulatory requirements and contractual agreements. CTD Inc. will ensure secure long-term storage and accessibility of data for future audit, inspection, or analysis, as specified in the study protocol and sponsor contract.

9.7. Data Analysis

All analysis will follow a pre-specified Statistical Analysis Plan (SAP), which will describe the statistical methodology, handling of missing data, and sensitivity analyses in detail. Statistical analyses will be conducted using validated software (e.g., SAS, R, or Stata), and two-sided p-values <0.05 will be considered statistically significant unless otherwise specified.

Analysis population

The analysis will include pediatric IPD cases meeting the following criteria:

- *S. pneumoniae* isolated from a normally sterile site (e.g., blood, cerebrospinal fluid, pleural fluid, peritoneal fluid, pericardial fluid, joint fluid).
- Isolate submitted for study testing, enabling serotyping and genomic characterization.
- Basic clinical data obtained during routine care (e.g., age, sex, underlying conditions, vaccination history, diagnosis, outcome) and registered in the EDC system.

All isolates are derived from routine clinical testing and would otherwise be discarded. Only non-identifiable clinical information will be used, and individual informed consent will not be required, in accordance with institutional ethical approvals.

Trend and comparative analyses

- Assess temporal trends in vaccine-type (VT) and non-vaccine-type (NVT) serotypes using the Cochran–Armitage trend test.
- Compare age-specific and vaccination-specific distributions using chi-square or Fisher's exact tests.

Exploratory analyses

- Summarize associations between clinical outcomes, underlying conditions, vaccination status, serotype, and resistance patterns descriptively.
- Apply logistic regression models in exploratory analyses to identify potential risk factors for infection with specific serotypes or resistant strains.

Missing data

Analyses will be performed on all available cases. Cases with missing key variables will be excluded from specific analyses, and sensitivity analyses will be detailed in SAP.

9.8. Descriptive analysis

A descriptive analysis will be conducted for all IPD cases identified from the years 2026 to 2028. Cases will be summarized by demographic variables (e.g., age group, sex), clinical features (e.g.,

hospitalization status, diagnosis, outcomes), and relevant clinical history (e.g., comorbid conditions, pneumococcal vaccination status).

For the distribution of pneumococcal capsular serotypes-the per cent (%) of each serotype will be calculated, with the denominator of all IPD isolates. Stratified analyses will be performed based on patient characteristics, including age group. Annual serotype-specific isolation rates will also be reported.

For antimicrobial susceptibility-the proportion (%) of isolates classified as susceptible, intermediate, or resistant to each tested antibiotic will be calculated based on CLSI breakpoints. Trends in resistance patterns will be evaluated across the study period.

Additional exploratory analyses may assess associations between serotypes or antimicrobial resistance profiles and clinical characteristics such as comorbidities, outcomes, and vaccine status including pneumococcal vaccination history. Vaccine coverage data will be obtained, and trends in the isolation rates of VT versus NVT serotypes over time (by year) will be examined to assess the impact of PCV15 and PCV20 introduction.

Serotype Distribution

The proportion of each pneumococcal serotype causing IPD will be calculated by dividing the number of IPD cases attributed to each specific serotype by the total number of IPD cases, excluding non-typeable (NT) isolates. Serotype distributions will be analyzed across different strata, including pre- and post-introduction periods of PCV20, age groups, and risk condition status (with/without high-risk conditions).

For analytical purposes, serotypes will be grouped into the following seven categories:

1. VST13 (Vaccine Serotype 13): All serotypes included in PCV13, plus 6C due to known cross - protection by PCV13. (1, 3, 4, 5, 6A, 6B, 6C*, 7F, 9V, 14, 18C, 19A, 19F, 23F)
2. VST15 (Vaccine Serotype 15): All serotypes included in PCV15 (1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 22F, 23F, 33F)
3. VST15-13: The two serotypes included in PCV15 but not in PCV13. (22F, 33F)
4. VST20 (Vaccine Serotype 20): All serotypes included in PCV20. (1, 3, 4, 5, 6A, 6B, 7F, 8, 9V, 10A, 11A, 12F, 14, 15B, 18C, 19A, 19F, 22F, 23F, 33F)
5. VST20-13: The seven serotypes included in PCV20 but not in PCV13, optionally including 15C. (8, 10A, 11A, 12F, 15B, 22F, 33F)
6. VST20-15: The five serotypes included in PCV20 but not in PCV15. (8, 10A, 11A, 12F, 15B)
7. NVST: non PCV20 serotypes

*Note: Serotype 6C is included under VST13 due to established cross-protection conferred by PCV13.

Serotyping

Serotyping for all samples will be conducted by Quellung reaction using antisera of 45 serotypes (1, 2, 3, 4, 5, 6A, 6B, 6C, 6D, 7C, 7F, 8, 9N, 9V, 10A, 11A, 12F, 13, 14, 15A, 15B, 15C, 15F, 16F, 17F, 18C, 19A, 19F, 20, 21, 22F, 23A, 23B, 23F, 24, 24B, 24F, 28A, 29, 31, 33F, 34, 35B, 37, 38) as the standard testing. For the unidentified serotypes by standard testing, additional serotyping will be conducted by Quellung reaction using antisera of the different serotypes from initial 45 serotypes.

Antimicrobial susceptibility* [1]

Antimicrobial susceptibility testing for SP isolates will be conducted using the broth microdilution method according to Clinical and Laboratory Standards Institute (CLSI) guidelines. The minimum inhibitory concentrations (MICs) will be interpreted primarily using the latest CLSI breakpoints (M100, 2025 edition) to ensure clinical relevance. In addition, secondary analyses will be performed using the 2008 CLSI breakpoints to allow for comparison with historical surveillance data. Isolates with intermediate or full resistance will be classified as non-susceptible. Breakpoints for susceptibility (S) Intermediate (I), and Resistant (R) are defined as follows:

Antimicrobial Agent		Interpretive Categories and MIC Breakpoint, µg/mL		
		Susceptible (S)	Intermediate (I)	Resistant (R)
PCG	Penicillin Parenteral (nonmeningitis)	≤ 2	4	≥ 8
	Penicillin Parenteral (meningitis)	≤ 0.06	-	≥ 0.12
	Penicillin (oral penicillin V)	≤ 0.06	0.12-1	≥ 2
CTRX	Ceftriaxone (meningitis)	≤ 0.5	1	≥ 2
	Ceftriaxone (nonmeningitis)	≤ 1	2	≥ 2
MEPM	Meropenem	≤ 0.25	0.5	≥ 4
LVFX	Levofloxacine	≤ 0.25	4	≥ 8
EM	Erythromycin	≤ 0.25	0.5	≥ 1

VCM	Vancomycin	≤ 1	-	-
TMP/SMX	Trimethopri/ Sulfamethoxazole (1:19)	≤ 0.5/9.5	1/19-2/38	≥ 4/76

CLSI M100-ED35: 2025 (EM100 Connect - CLSI M100 ED35:2025)

* Antibiotics distributed in Japan by Pfizer will be NOT used as test reagents in antimicrobial susceptibility testing.

Multi-locus sequence typing (MLST)

MLST will be performed to determine the sequence types (STs) of SP isolates, following established protocols [10] . Clonal complexes (CCs) will be identified using the goeBURST algorithm [11], with a CC defined as a group of STs sharing identical alleles at six out of seven housekeeping loci (i.e., single locus variants).

Whole-genome sequencing analysis

Draft genome data will be obtained for all isolates collected in this study. Genomic DNA will be extracted using the QIAamp DNA Mini Kit (QIAGEN), followed by library preparation for Illumina short-read sequencing using the Nextera XT Library Prep Kit (Illumina). After sequencing, antimicrobial resistance gene detection, PBP typing, and pilus typing will be performed. If necessary (e.g., upon identification of a highly virulent clone), more detailed genomic analyses-such as Global Pneumococcal Sequence Cluster (GPSC), typing, phylogenetic analysis, and phylogeographic analysis-will be conducted.

9.9. Interim analysis

Interim activities will consist only of descriptive data summaries and operational monitoring aligned with the scheduled reporting milestones. No inferential statistics (e.g., hypothesis testing or model-based estimation) will be performed at interim time points. Interim outputs are intended for operational oversight and quality management and will not be used for decision-making or to draw study conclusions.

Study Progress Report: 30 November 2026
Interim Report 1: 30 November 2027
Interim Report 2: 30 November 2028
Final report: 30 November 2029

Scope of interim summaries

- Enrollment trends over time (weekly/monthly/annual) and site-level case counts
- Basic patient demographics and clinical characteristics (age group, sex, prefecture, diagnosis, specimen type)

- Specimen submission and laboratory processing status (isolate receipt rate; completion rates for serotyping, AST, MLST/WGS; turnaround times)
- Data quality monitoring (eCRF completeness, logic checks, discrepancy resolution)
- Preliminary descriptive findings (e.g., annual proportions of serotypes and resistance profiles)

Handling

- No hypothesis testing, regression modeling, or effect estimation will be conducted at interim stages.
- Table/figure specifications and visualizations will follow the SAP addendum and may be updated as needed.
- Any substantive change to the analysis approach will be documented and approved as an SAP amendment.
- Patterns observed in interim summaries will be treated as exploratory and reassessed in the final analysis.

9.10. Quality Control

Quality control procedures for this study will be conducted in accordance with the Quality Plan. This includes standardized procedures for data collection, specimen handling, microbiological testing, and data entry into the EDC system. Regular data monitoring and validation checks will be performed to ensure completeness, accuracy, and consistency of the collected information.

9.11. Limitations of the Research Methods

This study has several limitations. First, it is anticipated that during the study period, both PCV15 and PCV20 are available under the NIP, and children may receive different types of PCVs depending on their timing of vaccination. This may limit the ability to fully assess the long-term and differential impacts of each vaccine.
Second, the study is based on enhanced surveillance rather than a population-based design. While active case identification improves data quality, there is a risk of underreporting or selection bias, as not all cases of IPD may be captured through participating sites.

9.12. Other Aspects

10. PROTECTION OF HUMAN PARTICIPANTS

10.1. Patient Information

This study does not collect personally identifiable information such as names or addresses of the patients with IPD whom SP strains are registered. Each patient and their isolate will be registered using a unique study ID assigned at the time of strain submission, along with the name of the medical

institution. The linkage between the study ID and the individual's identity will be securely maintained by each participating medical institution and will not be shared externally.

All institutions and personnel involved in the study will strictly adhere to data protection regulations to safeguard patient confidentiality. Data and specimen information exchanged between the participating sites, testing laboratories, and the study coordination office will be managed through secure channels and will not involve any third-party access. These data protection and confidentiality obligations will also be incorporated into subcontracts or agreements between the lead site and all participating sub-sites, to ensure compliance across all study centers.

10.2. Patient Consent

In accordance with the Japanese Ethical Guidelines for Life Science and Medical Research Involving Human Subjects (Chapter 4, Section 8: Informed Consent Procedures), written informed consent is not required when using anonymized clinical specimens (such as bacterial isolates) and associated data collected through routine care, provided that these data do not include personally identifiable information.

In this study, only bacterial isolates (SP) and anonymized clinical information obtained through standard clinical care will be collected. No patient-identifiable information will be shared outside of each institution. Therefore, obtaining written or verbal informed consent from patients or guardians is not required.

Each participating site will ensure that the data shared are fully anonymized prior to transfer, in compliance with ethical and data protection standards.

This information will be made available on the following public website:

National Surveillance of Pneumococcal Infections in Children

(http://pneumocatch.jp/)

However, each site is responsible for retaining a copy of the study protocol and ensuring that data shared with the study coordination office are fully anonymized in accordance with ethical and data protection standards.

As stated in Section 11, all records related to the provision of existing samples and data must be retained for at least five years following study completion.

10.3. Institutional Review Board (IRB)/Ethics Committee (EC)

Before the initiation of this study at each site, the research protocol must be reviewed and approved by the central Ethics Review Committee of the representative institution (National Hospital Organization Mie National Hospital).

As this study is a multi-institutional collaborative research project, and in view of the need to promote its smooth implementation, participating institutions will not be required to obtain additional local IRB/EC review, provided that centralized review has been conducted. Instead, before initiating the study at each

site, written permission from the head of the participating institution must be obtained, in accordance with the Ethical Guidelines for Life Science and Medical Research Involving Human Subjects.

10.4. Ethical Conduct of the Study

This study will be conducted in accordance with the Ethical Guidelines for Life Science and Medical Research Involving Human Subjects (most recently revised in February 2023; hereinafter referred to as "the Guidelines"). The research protocol will be reviewed and approved by the Ethics Review Committee of the National Hospital Organization Mie National Hospital to ensure that the study complies with the Guidelines and addresses all necessary ethical considerations.

11. MANAGEMENT AND REPORTING OF ADVERSE EVENTS/ADVERSE REACTIONS

Although the study includes analysis related to Pfizer products, it does not involve the administration of any investigational or licensed product to study subjects. This is a secondary data collection study involving the review of structured data from existing medical records and microbiological samples. Individual safety reporting (e.g., adverse event or adverse reaction reporting) is applicable and will be required.

Safety Reporting Requirements are summarized below, but more detail is provided in the Safety Reporting Reference Manual for Investigator Sponsored Research (ISR) or Clinical Research Collaboration (CRC) Studies For Non-Interventional Studies (NIS).

Human Review of Unstructured Data

This study protocol requires human review of patient-level unstructured data. Unstructured data refer to verbatim medical data, including text-based descriptions and visual depictions of medical information, such as medical records, images of physician notes, neurological scans, x-rays, or narrative fields in a database. The reviewer is obligated to report adverse events (AEs) with explicit attribution to the Pfizer product(s) under study that appear in the reviewed information (defined per the patient population and study period specified in the protocol) to the Pfizer Drug Safety Unit (DSU). Explicit attribution is not inferred by a temporal relationship between drug administration and an AE but must be based on a definite statement of causality by a healthcare provider linking product administration to the AE.

The requirements for reporting safety events on the Investigator Sponsored Research (ISR)/Clinical Research Collaboration (CRC) NIS adverse event Report Form to Pfizer Safety are as follows:

- All serious and non-serious AEs with explicit attribution to the Pfizer product(s) under study that appear in the reviewed information must be reported, within 24 hours of awareness, to Pfizer Safety using the ISR/CRC NIS adverse event Report Form.
- Scenarios involving drug exposure, including exposure during pregnancy, exposure during breast feeding, medication error, overdose, misuse, extravasation, lack of efficacy (such as an instance of IPD despite prior PCV vaccination with a Pfizer product), and occupational exposure associated with the use of the Pfizer product(s) under study and must be reported, regardless if

there is an associated AE, within 24 hours of awareness, to Pfizer Safety using the ISR/CRC NIS adverse event Report Form.

- For these AEs with an explicit attribution or scenarios involving exposure to a Pfizer product, the safety information identified in the unstructured data reviewed is captured in the Event Narrative section of the report form, and constitutes all clinical information known regarding these AEs. No follow-up on related AEs will be conducted.
- The serotyping results will be reported to both the Principal Investigator (PI) and participating sites who registered the case. If the results indicate that the registered patient developed vaccine-type IPD (for those that received Pfizer products), the PI will check the clinical data collected in EDC and prepare and submit the ISR/CRC NIS adverse event Report form within 24 hours of awareness.

12. PLANS FOR DISSEMINATING AND COMMUNICATING STUDY RESULTS

The findings from this study will be disseminated through academic and professional channels. Results will be submitted for presentation at national and international scientific meetings. Additionally, at least one peer-reviewed manuscript will be prepared and submitted for publication.

13. AUTHORSHIP FOR PUBLICATION

We will follow the International Committee of Medical Journal Editors (ICMJE) criteria for authorship, which dictates that all authors must meet these criteria and all who meet these criteria must be authors. As a research collaboration it is anticipated that representatives from the principal investigators' institutions and Pfizer will participate in manuscripts, particularly those reporting primary study results. Researchers from the sites will also be considered to be authors if they meet ICMJE criteria. Each organization will need to determine who among its staff met ICMJE criteria, and the study PI will have responsibility for coordinating this assessment with local study teams.

14. REFERENCES

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