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Annual Report of the National Center for invasive Pneumococci (NZPn), 2022

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1. Organization

Since 1 March 2002, the Institute for Infectious Diseases, University of Bern hosts the National Center for invasive Pneumococci (NZPn) which is subsidized by the Federal Office of Public Health (FOPH). The overall objective of the center is a monitoring of the pneumococcal serotypes and antibiotic resistance rates from invasive *Streptococcus pneumoniae*. The NZPn is co-led by Dr. phil. nat. Carlo Casanova (diagnostics and administrative part) and PD. Dr. phil. nat. Markus Hilty (research part) under the supervision of Prof. Dr. med. Stephen Leib.

2. Diagnostics and quality assurance

Among the tasks of NZPn are confirmatory diagnostics of invasive pneumococci, serotyping and the analysis of relevant antibiotic resistance information. More specifically, the tasks include:

Table 1: Overview of the different tasks of the NZPn in diagnostics and quality assurance.

Routine and special tasks of the NZPn	Chapter Number
Confirmatory diagnostics/national monitoring of quality	2.1.1
Strain collection	2.1.2
Serotyping of invasive pneumococcal isolates	2.1.3
Antibiotic resistance data of invasive pneumococcal isolates	2.1.4
National and International quality assurance	2.1.5
Development of new diagnostic tools	2.1.6
Research	2.1.7

2.1.1 Confirmatory diagnostics/national monitoring of quality

- **Method**: In Switzerland, reporting of invasive pneumococcal disease (IPD) is mandatory, and the clinical pneumococcal isolates are sent by the diagnostic laboratories to the national reference center. The species identification of all strains submitted to the NZPn is verified by optochin susceptibility testing. As optochin resistant *S. pneumoniae* have been reported, isolates with reduced susceptibility are subjected to additional analysis (bile solubility, MALDI-TOF MS, in case of inconsistent results since 2021 whole genome sequencing (WGS)). The serogroup/serotype is determined by the Quellung reaction. In the absence of a reaction with a specific antiserum the isolate is reported as *S. pneumoniae* serotype 0 (i.e. non-typeable). Using WGS analysis these isolates are differentiated from optochin susceptible non-pneumococcal viridans streptococci.

- **Results**: In 2022 the NZPn received 971 isolates recovered from IPD. Thereof, 47 isolates were not *S. pneumoniae* or could not be cultured after transport, even after re-submission. Of the *S. pneumoniae* isolates 24 were excluded as duplicates (isolates of the same serotype isolated from the same patient within less than 5 days – usually from different body sites). Thus, in the final analysis 900 isolates were included. Thereof, 798 strains were isolated from blood, 24 from cerebrospinal fluid, 5 from pleural fluid, 3 from synovial fluid and 70 from other or not declared sites. The total annual numbers of IPD isolates significantly decreased in 2020 and 2021 due to the COVID-19 pandemic. The total number of isolates referred in 2022 was again similar to the years before the pandemic. (Figure 1 and Table 2). While the numbers were still low in January – March, the monthly numbers in November and December exceeded the numbers in previous years (Figure 2)
- The largest proportion of PCV13 and non-PCV13 isolates was recovered from IPD patients >65 years, followed by the age groups 50-65 years, 25-49 years and children <5 years (Figure 3). Relatively fewer isolates were found in older children and young adults.

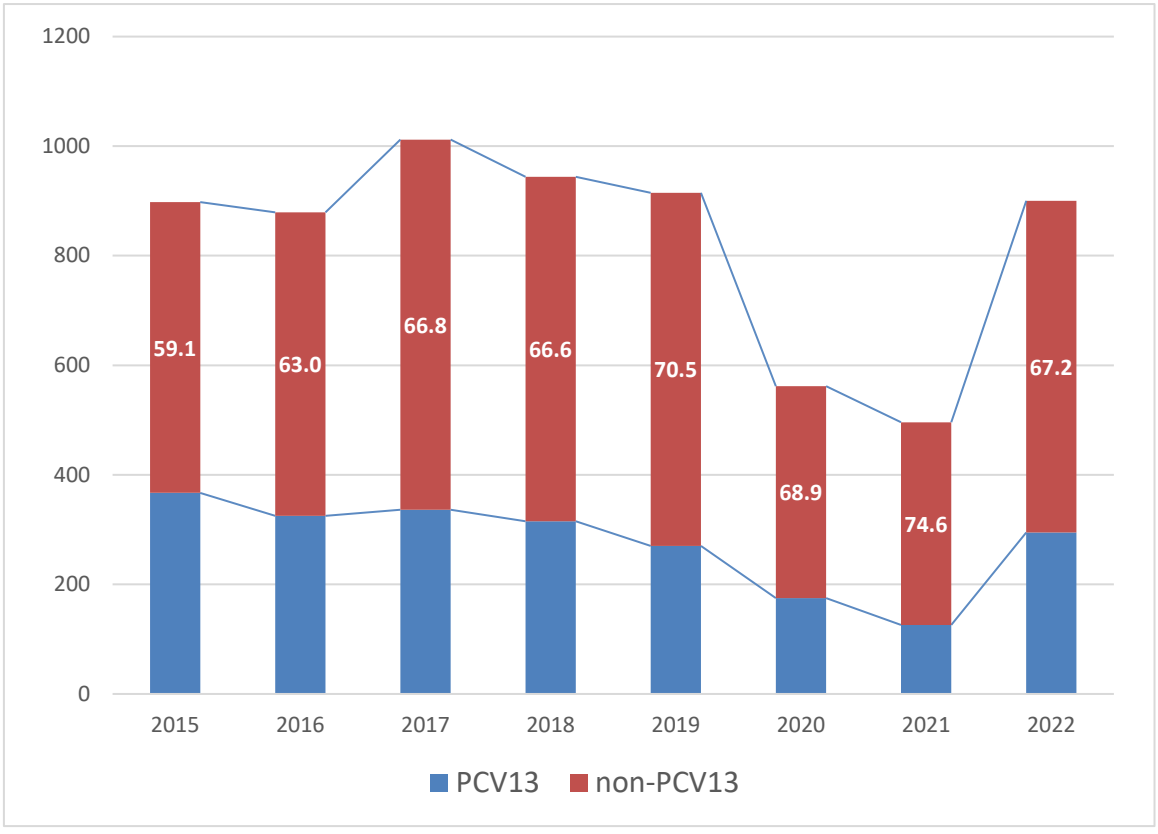


Figure 1 *Annual numbers of IPD isolates referred to the NZPn. The numbers in white indicate the proportion of non-PCV13 serotype isolates (%)*

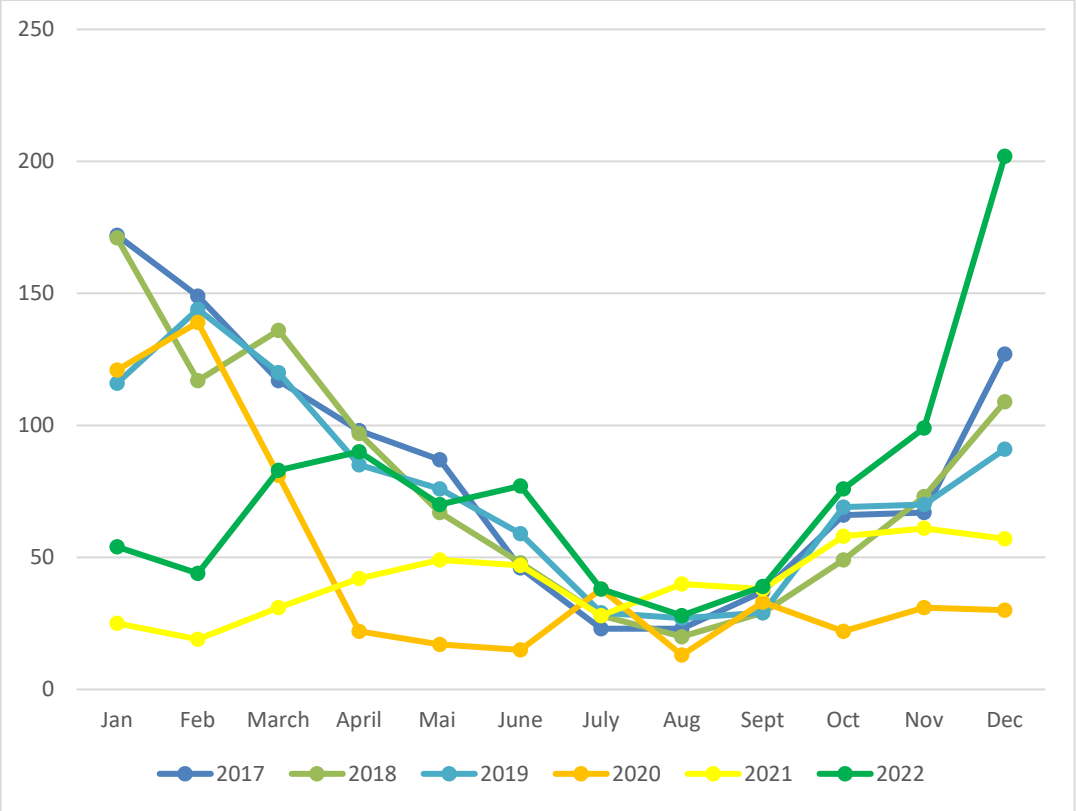


Figure 2 *Monthly numbers of IPD isolates referred to the NZPn*

- **Conclusion:** After a reduction in IPD isolates during the COVID-19 pandemic the number of IPD cases/isolates reached pre-pandemic levels again in March 2022. In November and December, we received even more isolates than in the years before in the same months.

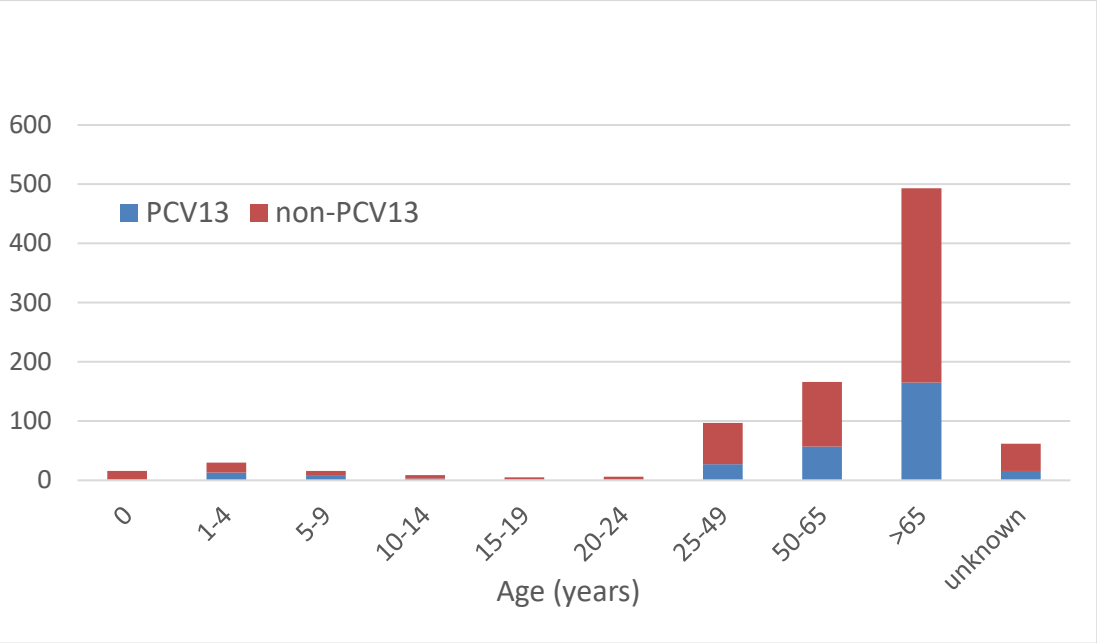


Figure 3 *Number of IPD isolates in 2022 by age group*

2.1.2 Strain collection

The NZPn stores all the received invasive pneumococcal isolates at -80°C. Collection and storage started in 2002 and currently includes more than 18'000 isolates. Biobanking of this large collection is currently being reorganized for compliance with standards of Swiss Biobank platform for storage and quality, comparability, accessibility, and interoperability of data.

2.1.3 Serotyping of invasive pneumococcal isolates

- **Introduction**: Since January 2011, the 13-valent pneumococcal conjugate vaccine (PCV13, Prevenar13®) has become available and has replaced PCV7 within the infant vaccine schedule in Switzerland. At the time of introduction, PCV13 covered a high percentage of circulating serotypes in Switzerland in all age groups. The previous experience with PCV7 suggests that PCV13 may induce a disappearance of PCV13 serotypes and cause emergence of non-vaccine serotypes.

- **Method**: After an isolate is confirmed as *S. pneumoniae*, its serogroup/serotype is determined with the Quellung reaction test (Neufeld test). In the absence of a reaction with any of the antisera, the isolate is reported as serotype 0 (i.e. non-typeable). The NZPn evaluates at the beginning of the year if new or additional pneumococcal antisera will be implemented in the diagnostic evaluation. This is because of the

introduction of PCV13, which leads to a redistribution of the serotype epidemiology. In 2022, no additional antisera were introduced. We currently test for the following serogroups/serotypes:

1	2	3	4	5	6	6A	6B	6C	7
7A	7F	8	9	9N	9V	10	10A	10B	11
11A	12	12A	12F	13	14	15A	15B*	15C*	15F
16A*	16F*	17A	17F	18	18C	18F	19	19F	19A
20	21	22	22F	23A	23B	23F	24A*	24B*	24F*
25	27	28	29	31	32	33	33A	33F	34
35	35B	35F	36	37	38	39	40	41	42
43	44	45	46	47	48				

*Additional factor sera to differentiate serotypes 15B and 15C, 16A and 16F, 24A, 24B and 24F were introduced in 2019.

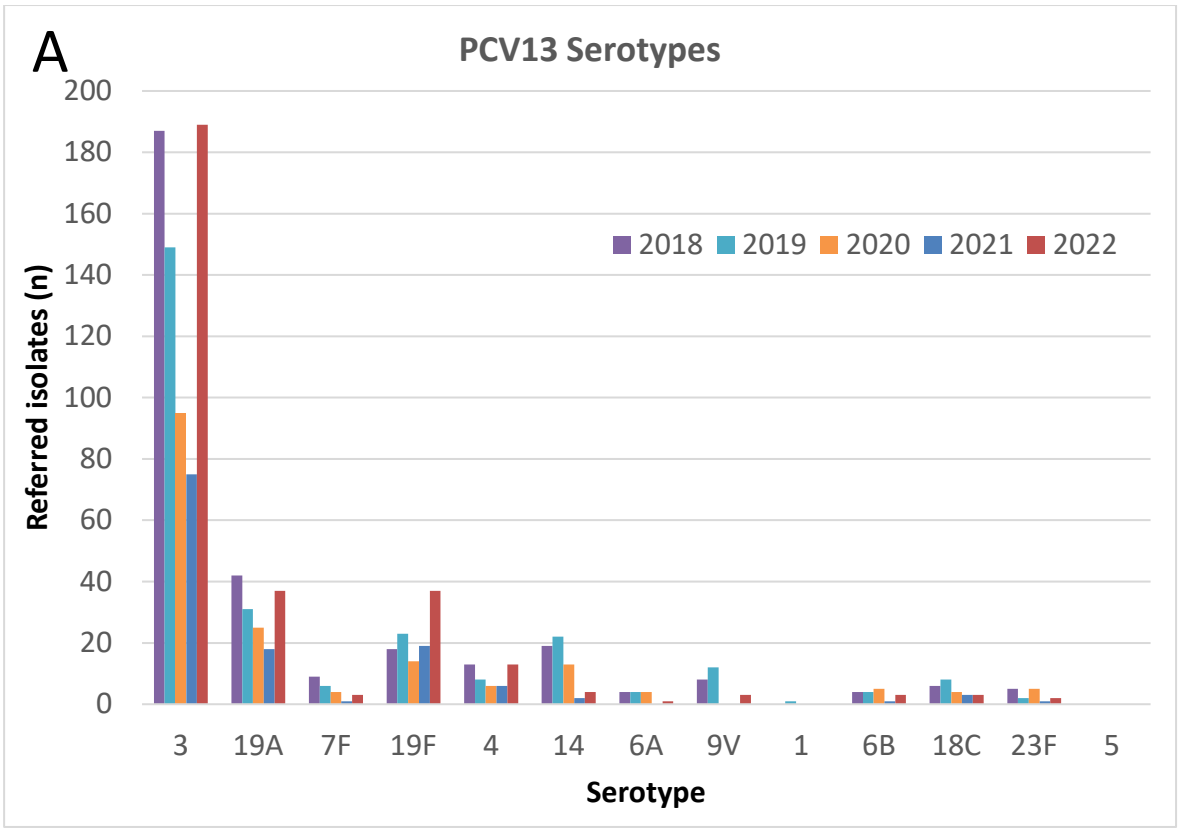
- **Results**: In 2022, the NZPn has received 900 non-duplicate strains of *Streptococcus pneumoniae* isolated from normally sterile body sites. In total 47 different serotypes/serogroups were identified.

  The six most frequent serotypes in 2022 were serotype 8 (n=194), serotype 3 (n=189), serotype 22F (n=66), serotype 9N (n=47) and serotypes 19A and 19F (both n=37) (Table 2 and Figure 4). Compared to 2021 there was no major change in the serotype distribution. The absolute numbers increased for most serotypes. Exceptions were e.g. serotypes 11A, 23B, 10B and 35B. The non-PCV13 serotype 8 is since 2020 the most frequent serotype, now being responsible for 21.6% of IPD cases in Switzerland (194 isolates in 2022). The numbers of non-PCV13 serotype 23B for which we reported an increase in 2021 (6% of the isolates, compared to 3.2% in 2020) [2] decreased again in 2022 (29 isolates, 3.2%). Among the vaccine type serotypes, the proportion of serotype 3 isolates again increased to 21% after a slight decrease was observed in the years before (from 19.8% in 2018 to 15.1 in 2021). Serotype 19F continued increasing to the same level as 19A (both 4.1% of isolates). In recent years the overall proportion of non-vaccine serotypes has continuously increased, replacing vaccine serotypes. In 2021 the proportion of non-PCV13 made almost three quarters (74.6%) of all IPD isolates. In 2022 it decreased again to a similar level as before the COVID-19 pandemic (67.2%).

  Conclusion: In 2022 there was no major change in the serotype distribution. The absolute numbers increased for most serotypes. The six most frequent serotypes in 2022 were the PCV13 serotypes 3, 19A and 19F and the non-vaccine serotypes 8 and 9N, together accounting for 63.3% of all IPD isolates. More than two thirds (67.2%) of IPD isolates were non-vaccine (PCV13) serotypes.

***Table 2**: Serotype distribution of referred IPD isolates 2018-2022. The five most frequent serotypes in each year are indicated in red. *Serogroup 16 exclusively consisted of serotype 16F isolates, serogroup 24 exclusively of 24F (n=23)), 15B/C consisted of 15B (n=14) and15C (n=6) isolates.*

	2018		2019		2020		2021		2022	
Serotype	n	%	n	%	n	%	n	%	n	%
3	**187**	**19.8**	**149**	**16.3**	**95**	**16.9**	**75**	**15.1**	**189**	**21.0**
19A	**42**	**4.4**	31	3.4	**25**	**4.4**	18	3.6	**37**	**4.1**
7F	9	1.0	6	0.7	4	0.7	1	0.2	3	0.3
19F	18	1.9	23	2.5	14	2.5	19	3.8	**37**	**4.1**
4	13	1.4	8	0.9	6	1.1	6	1.2	13	1.4
14	19	2.0	22	2.4	13	2.3	2	0.4	4	0.4
6A	4	0.4	4	0.4	4	0.7	0	0.0	1	0.1
9V	8	0.8	12	1.3	0	0.0	0	0.0	3	0.3
1	0	0.0	1	0.1	0	0.0	0	0.0	0	0.0
6B	4	0.4	4	0.4	5	0.9	1	0.2	3	0.3
18C	6	0.6	8	0.9	4	0.7	3	0.6	3	0.3
23F	5	0.5	2	0.2	5	0.9	1	0.2	2	0.2
5	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
Total PCV13	315	33.4	270	29.5	175	31.1	126	25.4	295	32.8
8	**160**	**16.9**	**142**	**15.5**	**96**	**17.1**	**114**	**23.0**	**194**	**21.6**
22F	**86**	**9.1**	**103**	**11.3**	**43**	**7.7**	**29**	**5.8**	**66**	**7.3**
9N	**50**	**5.3**	**63**	**6.9**	**33**	**5.9**	**29**	**5.8**	**47**	**5.2**
24*	13	1.4	13	1.4	12	2.1	8	1.6	23	2.6
15A	27	2.9	29	3.2	11	2.0	10	2.0	28	3.1
12F	37	3.9	**48**	**5.2**	13	2.3	7	1.4	13	1.4
10A	25	2.6	30	3.3	19	3.4	16	3.2	20	2.2
15B/C*	25	2.6	19	2.1	21	3.7	11	2.2	20	2.2
6C	15	1.6	13	1.4	10	1.8	8	1.6	10	1.1
11A	24	2.5	19	2.1	12	2.1	19	3.8	14	1.6
23B	33	3.5	23	2.5	18	3.2	**30**	**6.0**	29	3.2
23A	20	2.1	29	3.2	14	2.5	16	3.2	18	2.0
35F	16	1.7	20	2.2	12	2.1	12	2.4	23	2.6
31	6	0.6	7	0.8	6	1.1	4	0.8	6	0.7
38	6	0.6	11	1.2	5	0.9	1	0.2	4	0.4
16*	9	1.0	11	1.2	11	2.0	5	1.0	10	1.1
20	13	1.4	12	1.3	3	0.5	5	1.0	7	0.8
33F	16	1.7	13	1.4	11	2.0	9	1.8	11	1.2
17F	8	0.8	11	1.2	2	0.4	6	1.2	8	0.9
10B	4	0.4	3	0.3	4	0.7	7	1.4	5	0.6
35B	11	1.2	5	0.5	10	1.8	7	1.4	6	0.7
7	5	0.5	6	0.7	5	1.8	5	1.0	14	1.6
Other	20	2.1	15	1.6	16	3.7	12	2.4	29	3.2
Total non-PCV13	629	66.6	645	70.5	387	68.9	370	74.6	605	67.2
Total	**944**	100	**915**	100	**562**	100	**496**	100.0	**900**	100.0



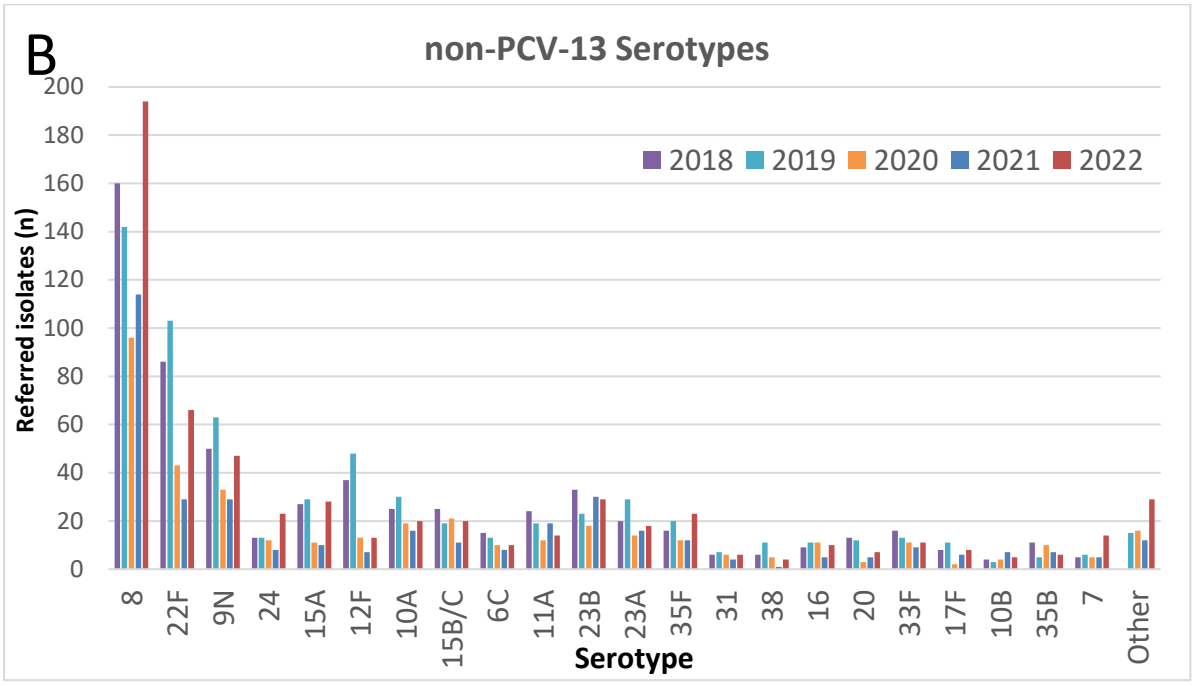


Figure 4 *Serotype distribution of invasive S. pneumoniae, annual absolute frequencies in 2018-2022 (A) PCV13 serotypes; (B) non-PCV13 serotypes*

2.1.4 Antibiotic resistance data of invasive pneumococcal isolates

- **Method**: Antibiotic susceptibility testing includes disk diffusion tests and, for isolates non-susceptible by oxacillin disk screen, minimal inhibitory concentration (MIC) determination by Etests®/Liofilchem® MIC test strips (bioMérieux, France and Liofilchem, Italy). Values determined on Mueller-Hinton Fastidious (MH-F) agar are interpreted according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines. Isolates susceptible by oxacillin disk screen or with a penicillin MIC ≤0.06 µg/mL are fully susceptible to penicillin and ceftriaxone irrespective of the clinical indication. For isolates with an MIC >0.06 µg/mL we report the MIC for penicillin and ceftriaxone (interpretive criteria shown in Table 3). Until December 2017 susceptibility testing was performed according to the American Clinical and Laboratory Standards Institute (CLSI) guidelines and, starting from January 2018, according to EUCAST guidelines.

Table 3: Interpretive standards for *S. pneumoniae* according to EUCAST v12.0. DD, disc diffusion; MIC, minimal inhibitory concentration; S, susceptible; R, resistant

Antimicrobial agent		EUCAST 2022	
		S	R
Penicilllin (oxacillin screen, all indications)	DD 1 µg oxacillin disc (mm)	≥20	
Penicillin parenteral (meningitis)	MIC (µg/ml)	≤0.06	>0.06
Penicillin parenteral (non-meningitis)	MIC (µg/ml)	≤0.06*	>2
Ceftriaxone (meningitis)	MIC (µg/ml)	≤0.5	>0.5
Ceftriaxone (non-meningitis)	MIC (µg/ml)	≤0.5	>2
Trimethoprim-sulfamethoxazole	DD 1.25/ 23.75 µg disc (mm)	≥13	<10
Erythromycin	DD 15µg disc (mm)	≥22	<19
Levofloxacin	DD 5µg (mm)	≥16**	<16

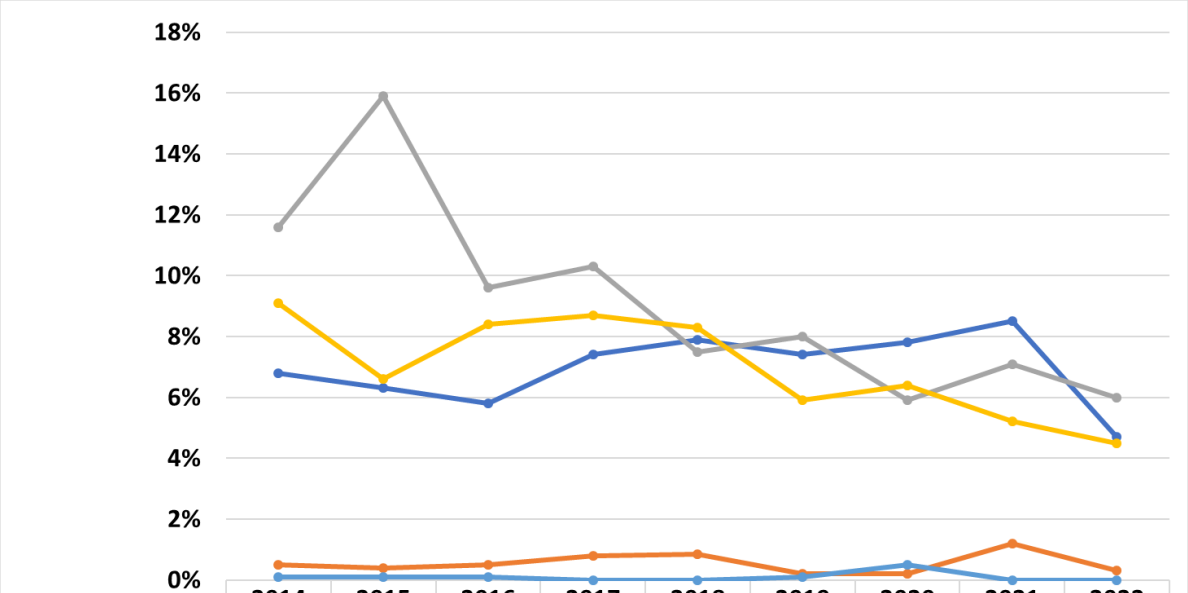
* For interpretation and dosing in pneumonia see

https://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Breakpoint_tables/v_12.0_Breakpoint_Tables.pdf

** "Susceptible, increased exposure (I)", http://www.eucast.org/clinical_breakpoints/

- **Results:** After a slight increase in the proportion of penicillin non-susceptible isolates in recent years (7.4% in 2019, 7.8% in 2020, 8.5% in 2021), In 2022 only 4.7% of the IPD isolates were resistant to penicillin according to meningitis

criteria (MIC >0.06 µg/mL, Figure 5). All isolates were susceptible by non-meningitis criteria (MIC ≤ 2 µg/ml). Three isolates (0.3%) were non-susceptible to ceftriaxone (resistant by meningitis criteria, intermediate by non-meningitis criteria). 4.5% of the isolates were non-susceptible (intermediate or resistant) to erythromycin and 6.0% to trimethoprim-sulfamethoxazole. All isolates were susceptible (increased exposure) to levofloxacin.



	2014	2015	2016	2017	2018	2019	2020	2021	2022
Penicillin	6.8%	6.3%	5.8%	7.4%	7.9%	7.4%	7.8%	8.5%	4.7%
Ceftriaxone	0.5%	0.4%	0.5%	0.8%	0.8%	0.2%	0.2%	1.2%	0.3%
Trim./Sulfa.	11.6%	15.9%	9.6%	10.3%	7.5%	8.0%	5.9%	7.1%	6.0%
Erythromycin	9.1%	6.6%	8.4%	8.7%	8.3%	5.9%	6.4%	5.2%	4.5%
Levofloxacin	0.1%	0.1%	0.1%	0.0%	0.0%	0.1%	0.5%	0.0%	0.0%

Figure 5 *Proportion of non-susceptible IPD isolates (% intermediate or resistant, does not include Levofloxacin EUCAST category I = susceptible increased exposure). For penicillin the meningitis interpretive criteria were applied.*

Conclusion: Compared to 2021, the resistance rate of IPD isolates in Switzerland decreased in 2022 for all tested antibiotics.

2.1.5 National and International quality assurance

The External Quality assurance (EQA) program organized by IBD-labnet / UK NEQAS scheduled for 2020 was postponed. Thus, no international quality assurance was carried out in 2022.

2.1.6 Research in development of new diagnostic tools

In 2022, we have published a study showing strain-level resolution and pneumococcal carriage dynamics by single-molecule real-time (SMRT) sequencing of the plyNCR marker [4]. We included a total of 872 nasal swab (NS) samples from 47 healthy infants during the first year of life. Pneumococcal carriage was determined using our newly developed SMRT sequencing methodology of the plyNCR marker. We found that the overall carriage prevalence was 63.8%, and pneumococcal co-colonization (≥ 2 plyNCR amplicon sequence variants (ASVs)) was detected in 38/213 (17.8%) sequenced samples. Therefore, our method was able to find and define co-colonization of *S. pneumoniae.*

2.1.7 Epidemiological Research

In collaboration, we assessed the association of serotype with case-fatality ratio (CFR) in invasive pneumococcal disease (IPD) and meningitis in South Africa, 2012-2018 (vaccine era), using multivariable logistic regression by manual backward elimination [5]. We found that, among meningitis patients of all ages, serotype 1 was associated with increased CFR. This has been part of a collaborative project with the university of the Witwatersrand, South Africa. The project is funded by the Swiss National Science Foundation (SNF http://p3.snf.ch/project-170844) and is led by Lucy Hathaway (Institute for Infectious Diseases, University of Bern) and Anne von Gottberg, (University of the Witwatersrand). In addition, as also already outlined in our report for 2021, we also take part at the IRIS network. A coincidence of invasive pneumococcal disease reduction with COVID-19 containment measures has been shown [1]. This collaboration has been further fostered and a new manuscript will be published in 2023.

2.1.8 Additional pneumococcal research at the NZPn

In 2022, we performed studies related to *Streptococcus pneumoniae* summarized below.

We identified peptide ligands which were able to suppress pneumococcal growth and that this effect was dependent on peptide length [6]. Growth was suppressed for diverse pneumococci, including antibiotic-resistant strains, but not other bacterial species tested. Further investigation of the peptides is now pending.

We also assessed the therapeutic potential of a novel endolysin PlyAZ3aT in an infant rat model of ceftriaxone-resistant pneumococcal meningitis [7]. However, we found that PlyAZ3aT did not improve survival compared to PBS, while survival for vancomycin treated animals was 70% which is a significant improvement when compared to PBS or PlyAZ3aT ($p<0.05$ each). We concluded that PlyAZ3aT failed to cure the infection due to an inability to reach the cerebrospinal fluid (CSF).

Optimization of the galenic formulation e.g. using liposomes might enable crossing of the blood brain barrier (BBB) and improve treatment efficacy.

3. Advisory service and networking

3.1 Advisory service

On special request we conduct whole genome sequencing analyses of *Streptococcus pneumoniae* strains. We have established the wet lab and *in silico* work flow for such analyses. Sequencing and data analysis are accredited to ISO/IEC 17025. The inhouse sequencing facility is led by PD Dr. Alban Ramette at the institute for infectious diseases.

3.2 Networking

The Pneumococcal Serotype Replacement and Distribution Estimation (PSERENADE) Project: In 2029, WHO commissioned the Pneumococcal Serotype Replacement and Distribution Estimation (PSERENADE) project to summarize the impact of PCV10/13 programs on IPD incidence and serotype distribution among children and adults. There are so far three manuscripts [8-10]. The collaboration has been ongoing in 2022 and more manuscripts are expected in the near future.

IRIS network: As mentioned above. A new manuscript will be published in 2023.

Murdoch Childrens Research Institute, Australia: A research collaboration is taking place in order to serotype interesting and 'difficult' strains. We will perform structural H-NMR analyses

4. Transfer of results

4.1 Transfer of data to the Federal Office of Public Health (FOPH)

The data collected in 2022 were sent to the FOPH on March 8, 2023.

4.2. Transfer of results to the referring laboratories

Serotyping and antimicrobial susceptibility testing results are usually sent to the referring laboratories within one week at the most. No irregularities have occurred during 2022.

5. Reporting

This report includes data of the NZPn from 2022. They are not matched with the IPD notification data of the FOPH. Therefore, results outlined in this report have to be interpreted with care.

6. Publications related to the topic within the reporting period (References)

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8. Bennett JC, Hetrich MK, Garcia Quesada M, Sinkevitch JN, Deloria Knoll M, Feikin DR, Zeger SL, Kagucia EW, Cohen AL, Ampofo K, et al: **Changes in Invasive Pneumococcal Disease Caused by Streptococcus pneumoniae Serotype 1 Following Introduction of PCV10 and PCV13: Findings from the PSERENADE Project.** *Microorganisms* 2021, **9**.
9. Deloria Knoll M, Bennett JC, Garcia Quesada M, Kagucia EW, Peterson ME, Feikin DR, Cohen AL, Hetrich MK, Yang Y, Sinkevitch JN, et al: **Global Landscape Review of Serotype-Specific Invasive Pneumococcal Disease Surveillance among Countries Using PCV10/13: The Pneumococcal Serotype Replacement and Distribution Estimation (PSERENADE) Project.** *Microorganisms* 2021, **9**.

10. Garcia Quesada M, Yang Y, Bennett JC, Hayford K, Zeger SL, Feikin DR, Peterson ME, Cohen AL, Almeida SCG, Ampofo K, et al: **Serotype Distribution of Remaining Pneumococcal Meningitis in the Mature PCV10/13 Period: Findings from the PSERENADE Project.** *Microorganisms* 2021, **9**.

Bern, 04.07.2023

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