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Annual Report of the National Center for invasive Pneumococci (NZPn), 2021

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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 2021 the NZPn received 535 isolates recovered from IPD. Thereof, 25 isolates were not *S. pneumoniae* or could not be cultured after transport, even after re-submission. Of the *S. pneumoniae* isolates 14 were excluded as duplicates (isolates of the same serotype isolated from the same patient within less than 4 days – usually from different body sites). Thus, in the final analysis 496 isolates were included. Thereof, 456 strains were isolated from blood, 12 from cerebrospinal fluid, 6 from pleural fluid, 6 from synovial fluid and 16 from other or not declared sites. The total annual numbers of IPD isolates significantly decreased in 2020 due to the COVID-19 pandemic. The number of isolates referred in 2021 was even slightly lower than in 2020. (Figure 1 and Table 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 2). Relatively fewer isolates were found in older children and young adults.

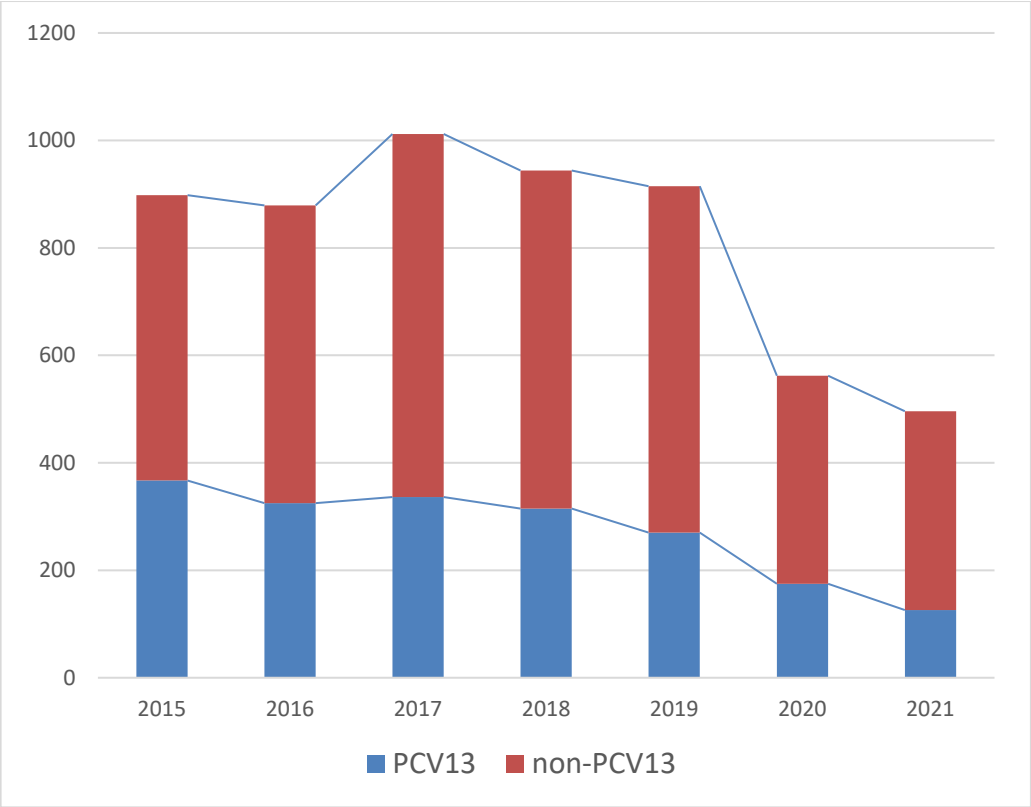


Figure 1 *Annual numbers of IPD isolates referred to the NZPn*

- **Conclusion:** During the COVID-19 pandemic the number of IPD cases decreased substantially. This decrease coincided with the stringency of containment measures in the pandemic [1]. Although the numbers of referred IPD isolates started to increase in the first half of 2021 after

loosening of COVID-19 measures [2], the total number of IPD isolates was even lower than in 2020.

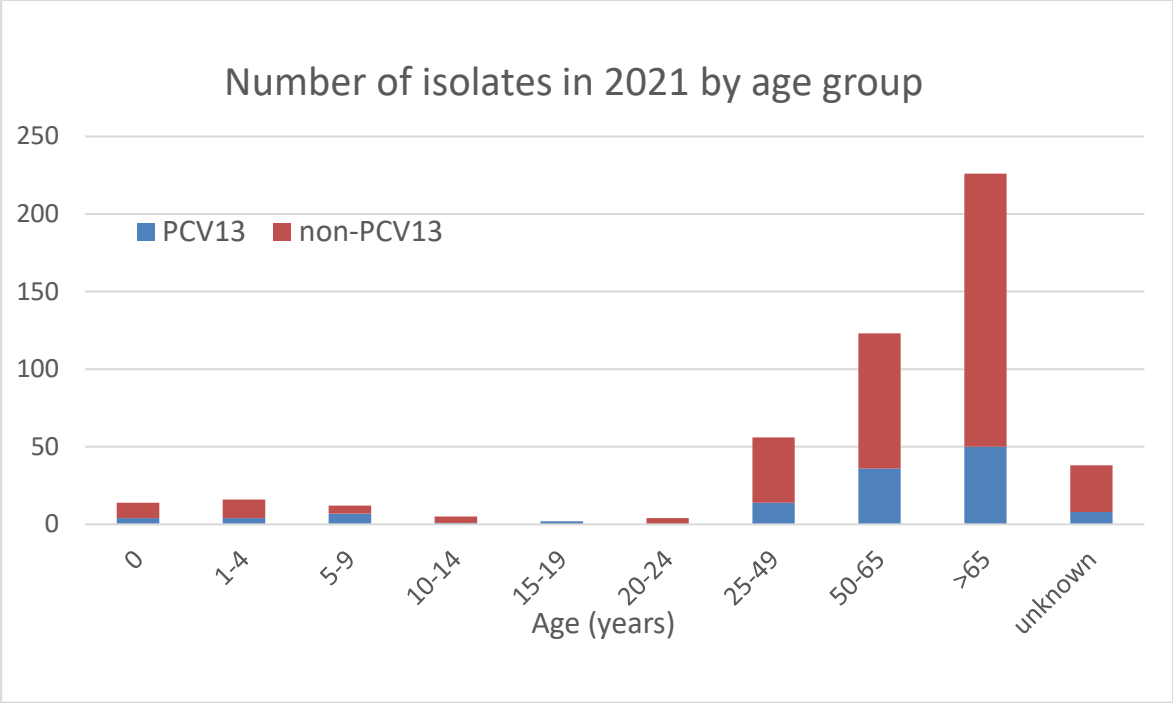


Figure 2 *Number of IPD isolates in 2021 by age group*

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 15'000 isolates. Biobanking of this large collection will be reorganized in the near future 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 very likely lead(s) to a redistribution of the serotype epidemiology. In 2020, 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				

  In 2019, we introduced additional factor sera to differentiate serotypes 15B and 15C, 16A and 16F, 24A, 24B and 24F. As we received fewer isolates in 2021 we had the capacity to retrospectively subtype isolates in these three serogroups (412 isolates from 2010-2018).

- **Results**: In 2021, the NZPn has received 496 non-duplicate strains of *Streptococcus pneumoniae* isolated from normally sterile body sites. In total 39 different serotypes/serogroups were identified.

  The five most frequent serotypes in 2021 were serotype 8 (n=114), serotype 3 (n=75), serotype 23B (n=30), 22F (n=29) and serotype 9N (n=29) (Table 2 and Figure 3). Compared to 2020 the distribution did not change much for most serotypes. The absolute numbers decreased slightly for most serotypes. Exceptions are serotype 8, 23B and 19F. The non-PCV13 serotype 8 is since 2020 the most frequent serotype, now being responsible for almost one quarter of IPD cases in Switzerland (23% of isolates in 2021). Surprisingly, the non-PCV13 serotype 23B has emerged (6% of the isolates, compared to 3.2% in 2020) and was the third most frequent serotype. Worryingly, serotype 23B is associated with reduced susceptibility to penicillin [2]. Among the vaccine type serotypes, 19F was the only serotype showing an increase in 2021 (from 2.5% in 2020 to 3.8%). There appeared to be a shift from serotype 19A (before 2021 among the five most frequent serotypes in Switzerland) to 19F isolates. In recent years the overall proportion of non-vaccine serotypes has continuously increased, replacing vaccine serotypes. In 2021 the proportion of non-PCV13 further increased and made almost three quarters (74.6%) of all IPD isolates

For the retrospectively subtyped isolates we found 15B and 15C both represented, while serogroups 16 and 24 almost exclusively consisted of 16F and 24F isolates.

Conclusion: In 2021 the total number of IPD isolates remained low when compared to the pre-pandemic years, and the distribution of individual serotypes did not change substantially. However, the proportion of non-vaccine serotypes worryingly, further increased, mainly due to increases in serotype 8 and 23B isolates.

***Table 2**: Serotype distribution of referred IPD isolates 2017-2021. The five most frequent serotypes in each year are indicated in red. *Serogroup 16 exclusively consisted of serotype 16F isolates, serogroup 24 of 24F (n=7) and 24B (n=1) isolates, 15B/C consisted of 15B (n=8) and15C (n=3) isolates.*

	2017		2018		2019		2020		2021	
Serotype	n	%	n	%	n	%	n	%	n	%
3	**158**	**15.6**	**187**	**19.8**	**149**	**16.3**	**95**	**16.9**	**75**	**15.1**
19A	**63**	**6.2**	**42**	**4.4**	31	3.4	**25**	**4.4**	18	3.6
7F	22	2.2	9	1.0	6	0.7	4	0.7	1	0.2
19F	27	2.7	18	1.9	23	2.5	14	2.5	19	3.8
4	21	2.1	13	1.4	8	0.9	6	1.1	6	1.2
14	17	1.7	19	2.0	22	2.4	13	2.3	2	0.4
6A	5	0.5	4	0.4	4	0.4	4	0.7	0	0.0
9V	6	0.6	8	0.8	12	1.3	0	0.0	0	0.0
1	3	0.3	0	0.0	1	0.1	0	0.0	0	0.0
6B	9	0.9	4	0.4	4	0.4	5	0.9	1	0.2
18C	1	0.1	6	0.6	8	0.9	4	0.7	3	0.6
23F	4	0.4	5	0.5	2	0.2	5	0.9	1	0.2
5	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
Total PCV13	336	33.2	315	33.4	270	29.5	175	31.1	126	25.4
8	**150**	**14.8**	**160**	**16.9**	**142**	**15.5**	**96**	**17.1**	**114**	**23.0**
22F	**91**	**9.0**	**86**	**9.1**	**103**	**11.3**	**43**	**7.7**	**29**	**5.8**
9N	**64**	**6.3**	**50**	**5.3**	**63**	**6.9**	**33**	**5.9**	**29**	**5.8**
24*	15	1.5	13	1.4	13	1.4	12	2.1	8	1.6
15A	31	3.1	27	2.9	29	3.2	11	2.0	10	2.0
12F	29	2.9	37	3.9	**48**	**5.2**	13	2.3	7	1.4
10A	24	2.4	25	2.6	30	3.3	19	3.4	16	3.2
15B/C*	24	2.4	25	2.6	19	2.1	21	3.7	11	2.2
6C	22	2.2	15	1.6	13	1.4	10	1.8	8	1.6
11A	27	2.7	24	2.5	19	2.1	12	2.1	19	3.8
23B	39	3.9	33	3.5	23	2.5	18	3.2	**30**	**6.0**
23A	27	2.7	20	2.1	29	3.2	14	2.5	16	3.2
35F	15	1.5	16	1.7	20	2.2	12	2.1	12	2.4
31	18	1.8	6	0.6	7	0.8	6	1.1	4	0.8
38	5	0.5	6	0.6	11	1.2	5	0.9	1	0.2
16*	15	1.5	9	1.0	11	1.2	11	2.0	5	1.0
20	17	1.7	13	1.4	12	1.3	3	0.5	5	1.0
33F	16	1.6	16	1.7	13	1.4	11	2.0	9	1.8
17F	8	0.8	8	0.8	11	1.2	2	0.4	6	1.2
10B	4	0.4	4	0.4	3	0.3	4	0.7	7	1.4
35B	14	1.4	11	1.2	5	0.5	10	1.8	7	1.4
Other	21	2.1	25	2.6	21	2.3	21	3.7	17	3.4
Total non-PCV13	676	66.8	629	66.6	645	70.5	387	68.9	370	74.6
Total	**1012**	100	**944**	100	**915**	100	**562**	100	**496**	100.0

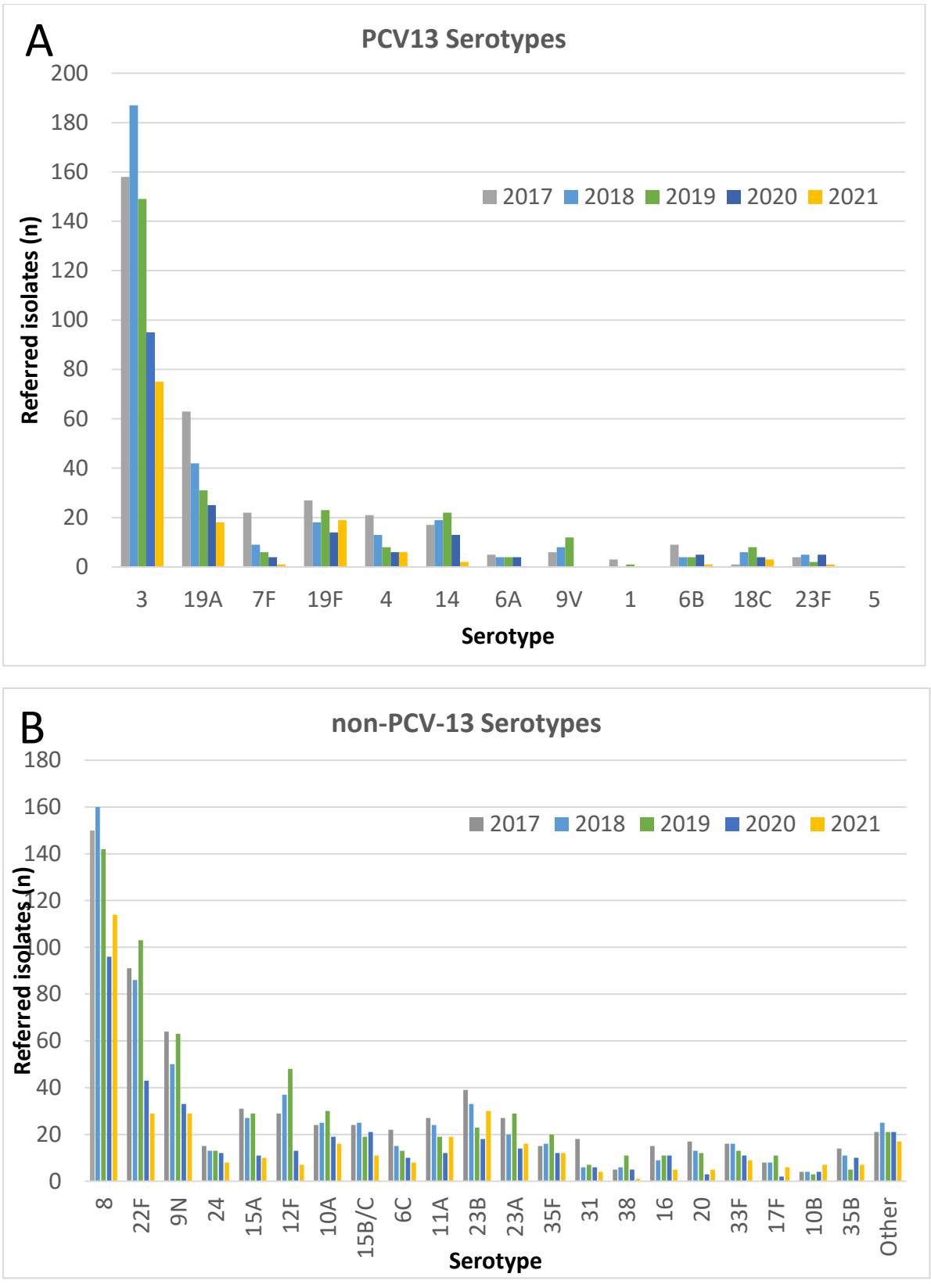


Figure 3 *Serotype distribution of invasive S. pneumoniae, annual absolute frequencies in 2017-2021 (A) PCV13 serotypes; (B) non-PCV13 serotypes*

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 v11.0. DD, disc diffusion; MIC, minimal inhibitory concentration; S, susceptible; R, resistant

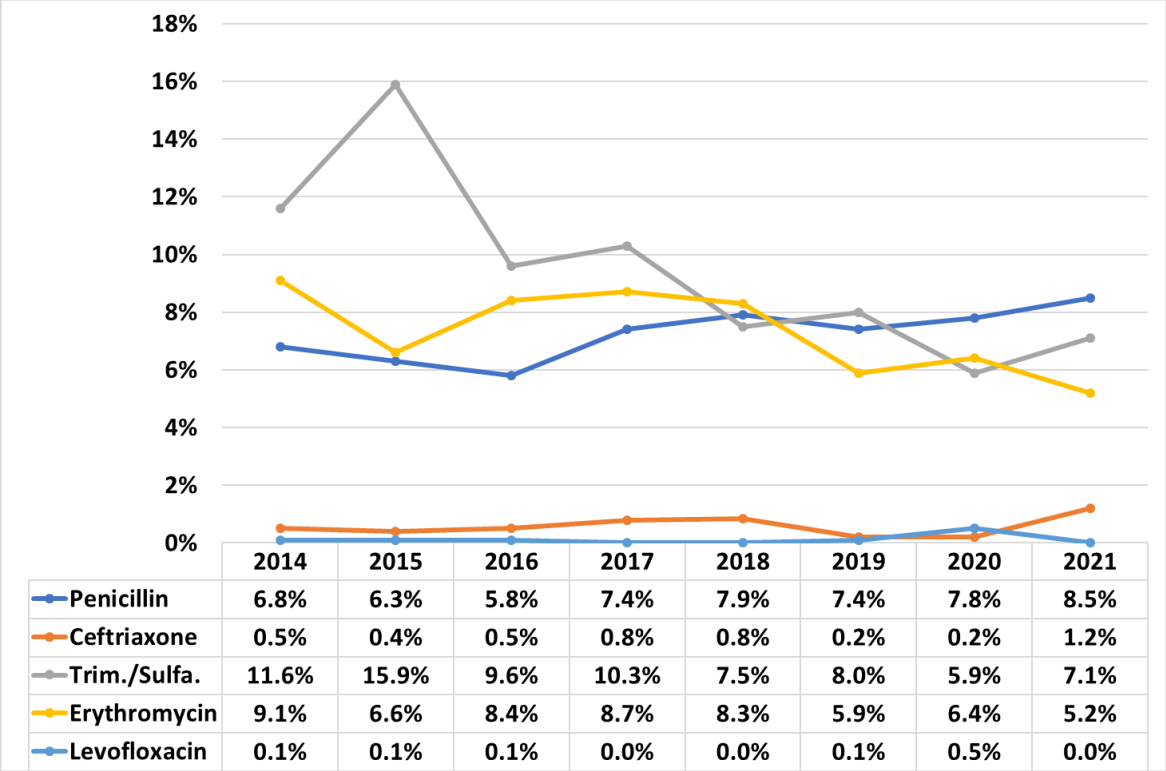
Antimicrobial agent		EUCAST 2021	
		S	R
Penicilllin (oxacillin screen, all indications)	DD 1 µg oxacillin disc (mm)	≥20	
Penicillin parenteral (meningitis)	MIC (µg/ml)	≤0.06	>0.06
Penicillin parenteral (non-meningitis)	MIC (µg/ml)	≤0.06*	>2
Ceftriaxone (meningitis)	MIC (µg/ml)	≤0.5	>0.5
Ceftriaxone (non-meningitis)	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/Dosages_v_11.0_Breakpoint_Tables.pdf

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

- **Results:** In 2021, 8.5% of the IPD isolates were resistant to penicillin according to meningitis criteria (MIC >0.06 µg/mL). The proportion of penicillin

non-susceptible isolates was slightly increasing in recent years (7.4% in 2019, 7.8% in 2020; Figure 4). All but one isolate was, however, susceptible by non-meningitis criteria (MIC ≤ 2 µg/ml). The proportion of IPD isolates non-susceptible to ceftriaxone (resistant by meningitis criteria, intermediate by non-meningitis criteria) increased from 0.2% to 1.2%. Four of the six non-susceptible isolates were of serotype 19F. As this serotype is associated with higher resistance, the increase in the overall resistance rate to ceftriaxone could be explained by the increased frequency of this serotype in 2021. 5,2% of the isolates were non-susceptible (intermediate or resistant) to erythromycin and 7.1% to trimethoprim-sulfamethoxazole. All isolates were susceptible (increased exposure) to levofloxacin.



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

Figure 4 *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: The resistance rate of IPD isolates in Switzerland increased in 2021 for penicillin, ceftriaxone and trimethoprim-sulfamethoxazole. This increase could be associated with the observed increase in the more resistant serotypes 23B and 19F [3].

2.1.5 National and International quality assurance

No international quality assurance was carried out in 2021. The External Quality assurance (EQA) program organized by IBD-labnet / UK NEQAS scheduled for 2020 was postponed.

2.1.6 Research in development of new diagnostic tools

In 2021, we have published a study showing differences in the capsular structure of serotype 6F strains using glucose as compared to galactose as the carbon source {Werren, 2021 #1593}. However, this study has already been mentioned in the report for 2020. We did not publish an additional manuscript for this section in 2021.

2.1.7 Epidemiological Research

As mentioned above we performed very recent epidemiological analyses to investigate the loosening of COVID-10 measure on the epidemiology of *Streptococcus pneumoniae*. We found that the IPD numbers again increased in Switzerland during the first six months of 2021 and that this coincides with the loosening of COVID-19 measures [2]. Vaccine pneumococcal serotypes have continued to decrease and non-vaccine type serotype 23B has emerged (8% of the isolates in 2021). Worryingly, serotype 23B is associated with reduced susceptibility to penicillin. As also already outlined in our report for 2020, 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 is foreseen in 2022.

2.1.8 Additional pneumococcal research at the NZPn

In 2021, we also performed some other studies related to *Streptococcus pneumoniae*. We measured 41 cytokines/chemokines and growth factors in cerebrospinal fluid (CSF) samples from 57 South African meningitis patients (collected in the period 2018-2019), with confirmed *S. pneumoniae* serotypes, using a multiplexed bead-based immunoassay [4]. Our data provided average CSF concentrations of a range of cytokines and growth factors for 18 different serotypes to serve as a basis for future studies investigating host-pathogen interaction during pneumococcal meningitis. We noted that differences in induction of IL-8 between serotypes may be particularly worthy of future study. 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). Its focus is the investigation of the virulence of pneumococcal serotypes in human meningitis. We also collaborate on a project which has further developed thee liposomal nanotrap technology to simultaneously neutralize the

whole palette of cytolysins produced by *Streptococcus pneumoniae*, *Streptococcus pyogenes* and *Streptococcus dysgalactiae* pathogens that can cause life-threatening streptococcal toxic shock syndrome [5]. We show that the mixture of liposomes containing high amounts of cholesterol and liposomes composed exclusively of choline-containing phospholipids is fully protective against the combined action of exotoxins secreted by these pathogens.

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 sequencing facility is led by PD Dr. Alban Ramette at our institute.

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 [6-8]. The collaboration has been ongoing in 2021 and more manuscripts are expected in the near future.

IRIS network: As mentioned above.

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

4. Transfer of results

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

The data collected in 2021 were sent to the FOPH on January 20, 2022.

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.

5. Reporting

This report includes data of the NZPn from 2021. 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)

1. Brueggemann AB, Jansen van Rensburg MJ, Shaw D, McCarthy ND, Jolley KA, Maiden MCJ, van der Linden MPG, Amin-Chowdhury Z, Bennett DE, Borrow R, et al: **Changes in the incidence of invasive disease due to Streptococcus pneumoniae, Haemophilus influenzae, and Neisseria meningitidis during the COVID-19 pandemic in 26 countries and territories in the Invasive Respiratory Infection Surveillance Initiative: a prospective analysis of surveillance data.** *Lancet Digit Health* 2021, **3:**e360-e370.
2. Casanova C, Kuffer M, Leib SL, Hilty M: **Re-emergence of invasive pneumococcal disease (IPD) and increase of serotype 23B after easing of COVID-19 measures, Switzerland, 2021.** *Emerg Microbes Infect* 2021, **10:**2202-2204.
3. Hauser C, Kronenberg A, Allemann A, Muhlemann K, Hilty M: **Serotype/serogroup-specific antibiotic non-susceptibility of invasive and non-invasive *Streptococcus pneumoniae*, Switzerland, 2004 to 2014.** *Eurosurveillance* 2016, **21:**12-21.
4. Muller A, Schramm DB, Kleynhans J, de Gouveia L, Meiring S, Ramette A, von Gottberg A, Hathaway LJ: **Cytokine response in cerebrospinal fluid of meningitis patients and outcome associated with pneumococcal serotype.** *Sci Rep* 2021, **11:**19920.
5. Besancon H, Babiychuk V, Larpin Y, Koffel R, Schittny D, Brockhus L, Hathaway LJ, Sendi P, Draeger A, Babiychuk E: **Tailored liposomal nanotraps for the treatment of Streptococcal infections.** *J Nanobiotechnology* 2021, **19:**46.
6. 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**.
7. 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**.
8. 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, 31 August 2022

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