Institute for Infectious Diseases, CH-3010 Bern

Institute for Infectious Diseases

Annual Report of the National Center for invasive Pneumococci (NZPn), 2020

Address

National Center for invasive Pneumococci (NZPn)
Institute for Infectious Diseases, University of Bern
Friedbühlstrasse 51
CH-3010 Bern, Switzerland
Phone ++41 31 632 32 65

Website: German:

http://www.ifik.unibe.ch/dienstleistungen/pneumokokken_zentrum/index_ger.html

Website: French:

https://www.ifik.unibe.ch/dienstleistungen/centre_national_pour_les_pneumocoques_invasifs_mandat_par_l_ofspa/index_ger.html

Carlo Casanova Tel. +41 (0)31 632 87 78 carlo.casanova@ifik.unibe.ch
Markus Hilty Tel. +41 (0)31 632 49 83 markus.hilty@ifik.unibe.ch
Stephen L. Leib Tel. +41 (0)31 632 49 49 stephen.leib@ifik.unibe.ch

Friedbühlstrasse 51
CH-3010 Bern www.ifik.unibe.ch

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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 DNA Probe analysis (AccuProbe *Streptococcus pneumoniae* culture identification test, (Gen-Probe, Inc.) or, as of 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 molecular

analysis (DNA probe or WGS, see above) these isolates are differentiated from optochin susceptible non-pneumococcal viridans streptococci.

- **Results**: In 2020 the NZPn received 593 isolates recovered from IPD. Thereof, 17 isolates were not *S. pneumoniae* or could not be cultured after transport. 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 562 isolates were included. Thereof, 519 strains were isolated from blood, 15 from cerebrospinal fluid, 9 from pleural fluid, 2 from synovial fluid and 17 from other or not declared sites. Compared to previous years, the total number of IPD isolates is significantly lower (Figure 1 and Table 1). In an international study by the Invasive Respiratory Infection Surveillance (IRIS) network, to which the NZPn contributed, revealed a coincidence of this reduction with COVID-19 containment measures (Figure 2) [1]. The same was observed for invasive *Haemophilus influenzae* and *Neisseria meningitidis* cases, but not the non-respiratory pathogen *Streptococcus agalactiae*.
- The largest proportion of PCV13 and non-PCV13 isolates was recovered from IPD patients >65 years, followed by the age groups 50-65 years and 25-49 years (Figure 3). Relatively fewer isolates were found in children and young adults.

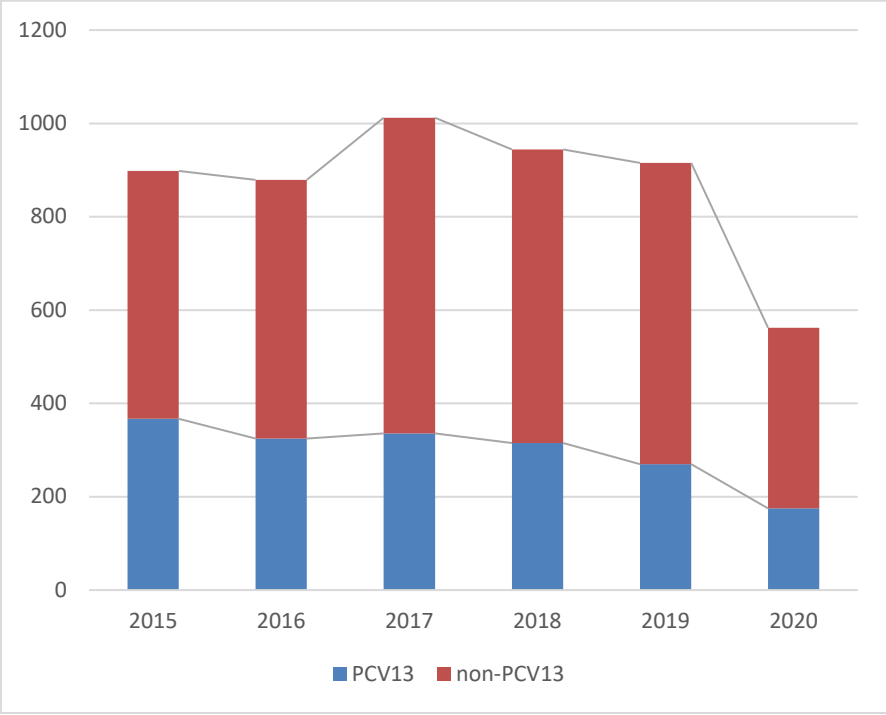


Figure 1 *Annual numbers of IPD isolates referred to the NZPn*

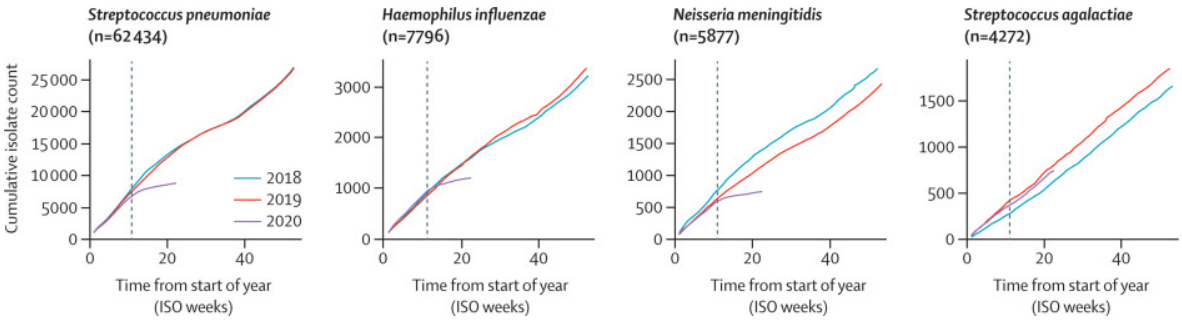


Figure 2 *Cumulative number of invasive isolates collected by surveillance laboratories in 26 countries. The dotted line indicates the time when WHO officially declared the COVID-19 pandemic [1]. (From Brueggemann et al. Lancet Digit Health. 2021 Jun;3(6):)*

- **Conclusion:** In 2020 we received the lowest annual number of invasive *S. pneumoniae* isolates since the beginning of acquisition of data at the NZPn in 2002.This could be explained by a reduced transmission of Pneumococci due to containment measures in the COVID-19 pandemic. Additionally, more indirect effects, such as fewer viral infections predisposing for bacterial superinfections or an increased willingness to seek early medical attention, could also have contributed to a reduction of IPD.

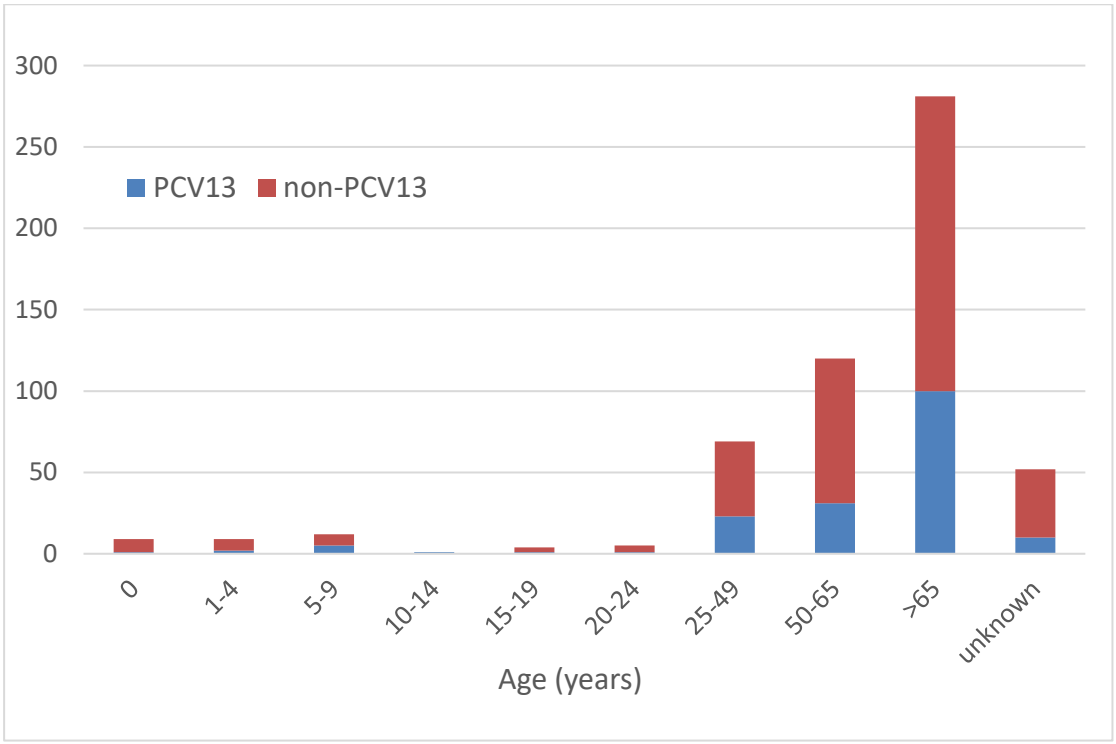


Figure 3 *Number of IPD isolates in 2020 by age group*

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 needs to be reorganized in the near future for compliance with standards of Swiss Biobank platform for storage and quality, comparability, accessibility, and interoperability of data. The FOPH will be invited to participate in the planning and support of the future biobank solutions.

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				

- **Results**: In 2020, the NZPn has received 562 non-duplicate strains of *Streptococcus pneumoniae* isolated from normally sterile body sites. In total 43 different serotypes/serogroups were identified in 2020.

The five most frequent serotypes in 2020 were serotype 8 (n=96), serotype 3 (n=95), serotype 22F (n=43), serotype 9N (n=33) and serotype 19A (n=25) (Table 1 and Figure 4). Compared to 2019 the serotype distribution did not change much. For the PCV13 serotypes there was a slight increase of the proportion of serotype 19A (from 3.4 to 4.4%) and a decrease for serotype 9V (from 1.3% to 0). For the non-PCV13 serotypes there was a slight increase for serotypes 8 (from 15.5 to 17.1%), serotypes 15B/C (from 2.1 to 3.7%) and serotype 35B (from 0.5 to 1.8%). A slight decrease was recorded for the proportion of serotype 22F (from 11.3 to 7.7%), 9N (from 6.9 to 5.9%), 15A (from 3.2 to 2.0%) or 12F (from 5.2 to 2.3%). For all other serotypes the proportion stayed within a 1% range compared to the previous year. In recent years the overall proportion of non-vaccine serotypes has continuously increased, replacing vaccine serotypes. In 2020 the proportion of non-PCV13 isolates slightly decreased, but still made more than two thirds of all IPD isolates (68.9% compared to 70.5% in 2019).

Conclusion: Despite a significant reduction in total IPD isolates in 2020, the serotype distribution remained for most serotypes largely the same as in previous years. The proportion of non-vaccine serotype isolates did not further increase but, for the first time since the introduction of PCV13, the most frequent serotype was a non-vaccine type (Serotype 8, 17.1%)

***Table 1**: Serotype distribution of referred IPD isolates 2016-2020. The five most frequent serotypes in each year are indicated in red. *Serogroup 16 and 24 exlusively consisted of 16F and 24F isolates, 15B/C consisted of 12 15B and 9 15C isolates.*

	2016		2017		2018		2019		2020	
Serotype	n	%	n	%	n	%	n	%	n	%
3	**165**	**18.8**	**158**	**15.6**	**187**	**19.8**	**149**	**16.3**	**95**	**16.9**
19A	**46**	**5.2**	**63**	**6.2**	**42**	**4.4**	31	3.4	**25**	**4.4**
7F	26	3.0	22	2.2	9	1.0	6	0.7	4	0.7
19F	20	2.3	27	2.7	18	1.9	23	2.5	14	2.5
4	19	2.2	21	2.1	13	1.4	8	0.9	6	1.1
14	18	2.0	17	1.7	19	2.0	22	2.4	13	2.3
6A	7	0.8	5	0.5	4	0.4	4	0.4	4	0.7
9V	7	0.8	6	0.6	8	0.8	12	1.3	0	0.0
1	5	0.6	3	0.3	0	0.0	1	0.1	0	0.0
6B	5	0.6	9	0.9	4	0.4	4	0.4	5	0.9
18C	4	0.5	1	0.1	6	0.6	8	0.9	4	0.7
23F	3	0.3	4	0.4	5	0.5	2	0.2	5	0.9
5	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
Total PCV13	325	37.0	336	33.2	315	33.4	270	29.5	175	31.1
8	**107**	**12.2**	**150**	**14.8**	**160**	**16.9**	**142**	**15.5**	**96**	**17.1**
22F	**83**	**9.4**	**91**	**9.0**	**86**	**9.1**	**103**	**11.3**	**43**	**7.7**
9N	**56**	**6.4**	**64**	**6.3**	**50**	**5.3**	**63**	**6.9**	**33**	**5.9**
24	31	3.5	15	1.5	13	1.4	13	1.4	12	2.1*
15A	26	3.0	31	3.1	27	2.9	29	3.2	11	2.0
12F	24	2.7	29	2.9	37	3.9	**48**	**5.2**	13	2.3
10A	22	2.5	24	2.4	25	2.6	30	3.3	19	3.4
15B/C	22	2.5	24	2.4	25	2.6	19	2.1	21	3.7*
6C	21	2.4	22	2.2	15	1.6	13	1.4	10	1.8
11A	20	2.3	27	2.7	24	2.5	19	2.1	12	2.1
23B	20	2.3	39	3.9	33	3.5	23	2.5	18	3.2
23A	16	1.8	27	2.7	20	2.1	29	3.2	14	2.5
35F	16	1.8	15	1.5	16	1.7	20	2.2	12	2.1
31	12	1.4	18	1.8	6	0.6	7	0.8	6	1.1
38	12	1.4	5	0.5	6	0.6	11	1.2	5	0.9
16	11	1.3	15	1.5	9	1.0	11	1.2	11	2.0*
20	11	1.3	17	1.7	13	1.4	12	1.3	3	0.5
33F	11	1.3	16	1.6	16	1.7	13	1.4	11	2.0
17F	8	0.9	8	0.8	8	0.8	11	1.2	2	0.4
10B	4	0.5	4	0.4	4	0.4	3	0.3	4	0.7
35B	3	0.3	14	1.4	11	1.2	5	0.5	10	1.8
Other	18	2.0	21	2.1	25	2.6	21	2.3	21	3.7
Total non-PCV13	554	63.0	676	66.8	629	66.6	645	70.5	387	68.9
Total	**879**	100	**1012**	100	**944**	100	**915**	100	**562**	100

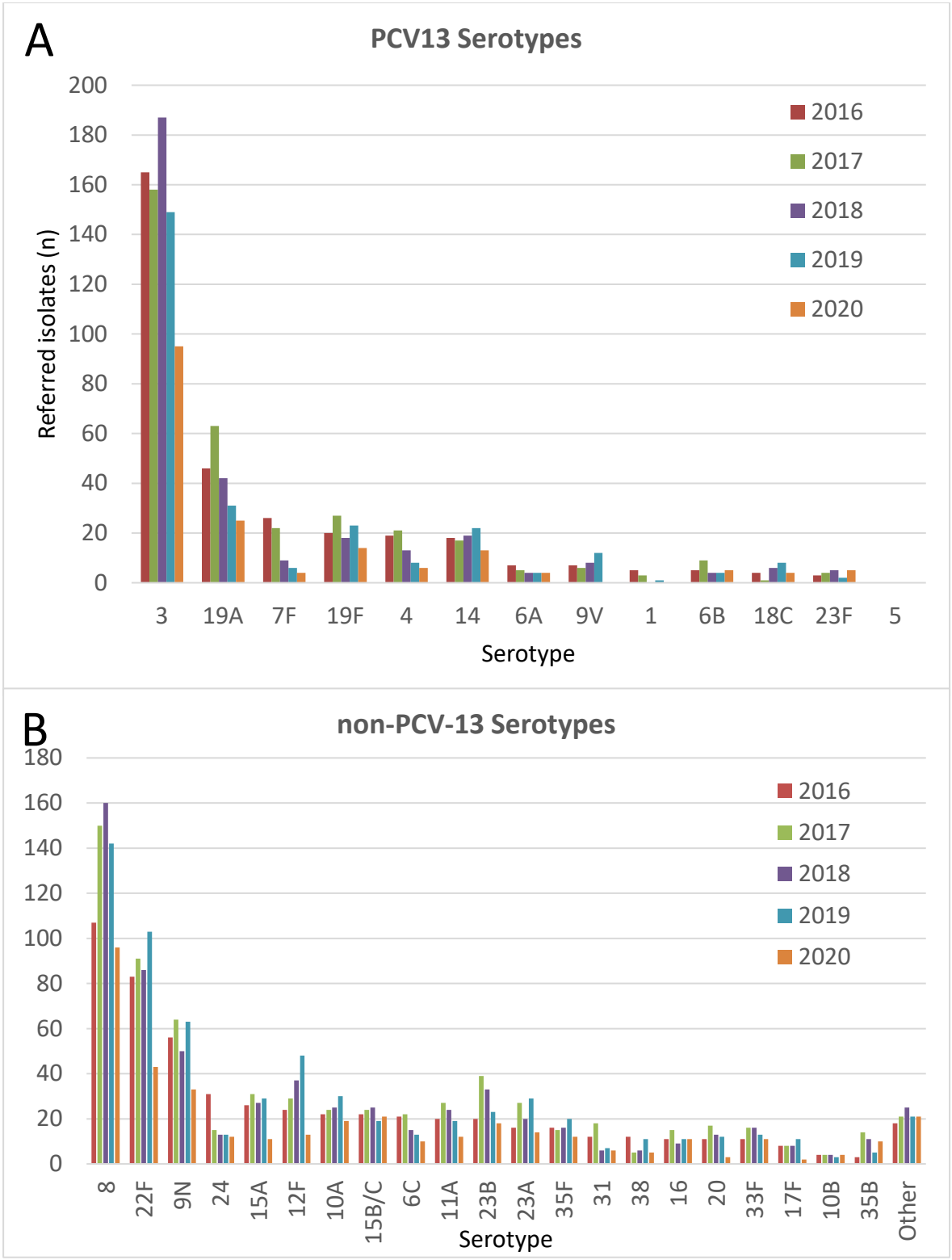


Figure 4 *Serotype distribution of invasive S. pneumoniae, annual absolute frequencies in 2016-2020 (A) PCV13 serotypes; (B) non-PCV13 serotypes*

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 v10.0. DD, disc diffusion; MIC, minimal inhibitory concentration; S, susceptible; R, resistant

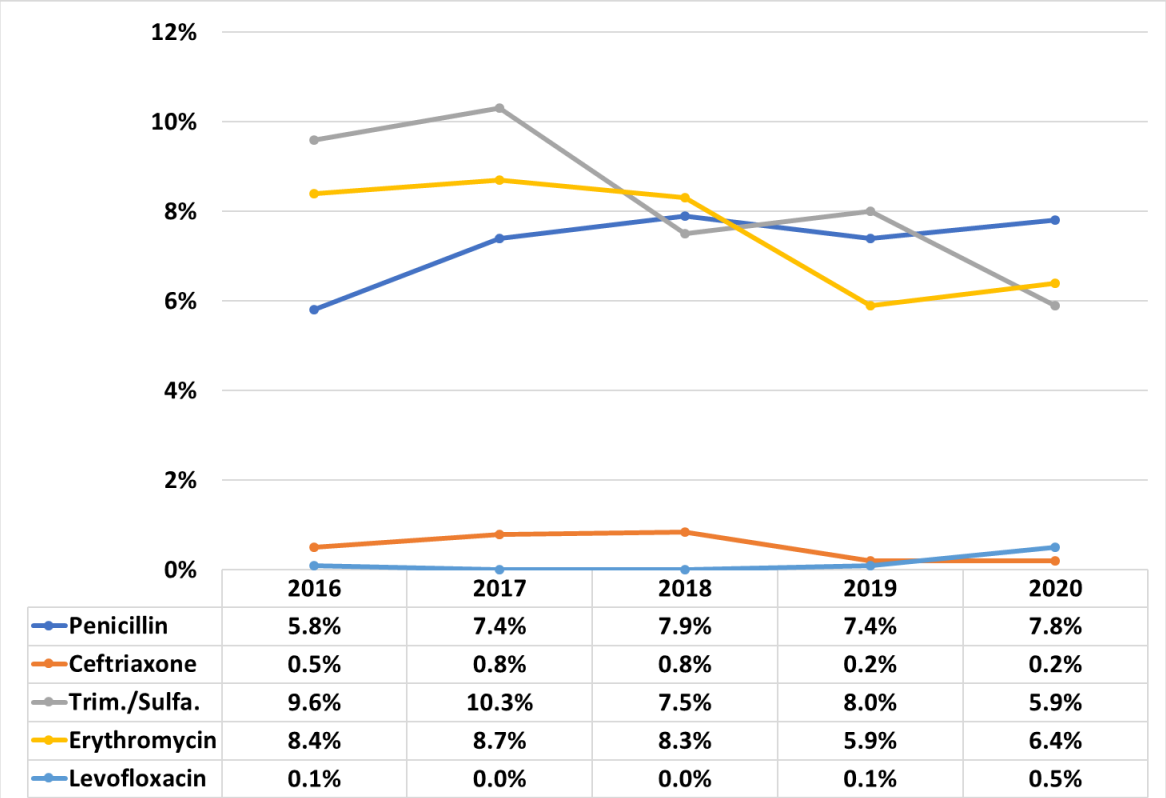
Antimicrobial agent		EUCAST 2020	
		S	R
Penicilllin (oxacillin screen. all indications)	DD 1 µg oxacillin disc (mm)	≥20	
Penicillin parenteral (meningitis)	MIC (µg/ml)	≤0.06	>0.06
Penicillin parenteral (non-meningitis)	MIC (µg/ml)	≤0.06*	>2
Ceftriaxone	MIC (µg/ml)	≤0.5	>2
Trimethoprim-sulfamethoxazole	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 2020, 7.8% of the IPD isolates were resistant to penicillin according to meningitis criteria (MIC >0.06 µg/mL). The proportion of penicillin non-susceptible isolates thus stayed on a similar level as in previous years (7.4% in 2019, 7.9% in 2018; Figure 5). All isolates were susceptible by non-meningitis criteria (MIC ≤ 2 µg/ml) and only one isolate was non-susceptible to

ceftriaxone (MIC 0.75 = intermediate; 0.2%). 6.4% of the isolates were non-susceptible (intermediate or resistant) to erythromycin and 5.9% to trimethoprim-sulfamethoxazole. Three isolates were resistant to levofloxacin (0.5%).



	2016	2017	2018	2019	2020
Penicillin	5.8%	7.4%	7.9%	7.4%	7.8%
Ceftriaxone	0.5%	0.8%	0.8%	0.2%	0.2%
Trim./Sulfa.	9.6%	10.3%	7.5%	8.0%	5.9%
Erythromycin	8.4%	8.7%	8.3%	5.9%	6.4%
Levofloxacin	0.1%	0.0%	0.0%	0.1%	0.5%

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:** The resistance rate of IPD isolates in Switzerland remained largely constant for most antibiotics tested. Compared to 2019 non-susceptibility slightly decreased for trimethoprim-sulfamethoxazole (from 8% to 5.9%) and increased for penicillin (from 7.4% to 7.8%), erythromycin (from 5.9% to 6.4%) and levofloxacin (0.1% to 0.5%).

2.1.5 National and International quality assurance

No international quality assurance was carried out in 2020. 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

We have recently investigated the environment-dependent composition of the polysaccharide structure of *S. pneumoniae* serotype 6F. When grown in a medium with glucose versus galactose, serotype 6F strains reveal a ratio of 1/0.6 or 1/0.3 for galactose/glucose in the capsule by ^{1}H-NMR, respectively. In line with this, flow cytometric experiments using monoclonal antibodies showed decreased labelling of Hyp6AG4 (specific for serotype 6A) antibodies when 6F is grown in glucose as compared to galactose. Therefore, this study shows differences in the capsular structure of serotype 6F strains using glucose as compared to galactose as the carbon source. In conclusion, 6F strains may show slightly different polysaccharide composition while using different growth media in a diagnostic laboratory. This may potentially complicate the serotyping for 6F strains [2].

2.1.7 Epidemiological Research

We recently examined the overall impact of both seven- and 13-valent PCVs (PCV7 and PCV13) on IPD in Switzerland from 2005-2019 [3]. We found that overall IPD incidence across all ages was only 16% lower in the late PCV13 era compared to the early PCV7 era (IRR 0.83, 95% CI 0.79-0.88), due to increasing incidence of non-PCV-type IPD (2.59, 2.37-2.83) in all age groups, except children <5 years. We also noticed that increased PCV resulted in a decrease in vaccine-type and overall IPD incidence across all age groups, in a regionally dependent manner. Taken together, the introduction of PCV13 has lowered overall IPD incidence, but the rising incidence of non-vaccine-type IPD, exclusive to older adults, may undermine indirect beneficial effects and needs close monitoring in the future [3].

As mentioned above, we also took part at the IRIS network. A coincidence of invasive pneumococcal disease reduction with COVID-19 containment measures has been shown [1]. The same was observed for invasive *Haemophilus influenzae* and *Neisseria meningitidis* cases, but not the non-respiratory pathogen *Streptococcus agalactiae*.

2.1.8 Additional pneumococcal research at the NZPn

In 2020, we have also continued and performed some studies related to pneumococcal meningitis (PM). We performed an evaluation of neurofilament light chain in the cerebrospinal fluid and blood as a biomarker for neuronal damage in experimental PM [4]. We also found that repetitive transcranial magnetic stimulation activates glial cells and inhibits neurogenesis after PM [5]. In another study we observed that adjuvant cannabinoid Receptor Type 2 agonist modulates the polarization of microglia towards a non-inflammatory phenotype in experimental PM [6]. Finally, we also published a study in which we suggest that pneumococcal serotype determines growth and capsule size in human

cerebrospinal fluid [7]. The latter 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.

3. Advisory service and networking

3.1 Advisory service

Molecular testing: On special request we conduct a molecular assay to determine the serotype directly from clinical IPD specimen. Our current Multiplex PCR approach covers 28 different serotypes/serogroups [8]. If a specific serotype is covered by the used Multiplex PCR, a result can usually be communicated to the clinicians.

3.2 Networking

Deutsches Referenzzentrum: We have collaborated with Mark van der Linden for a project to better characterize *S. pneumoniae* strains with serotype 6F [2]. We shared isolates and aimed at characterizing using NMR and whole genome sequencing methods.

IRIS network: As mentioned above.

Murdoch Childrens Research Institute, Australia: A research collaboration with the head of the microbiological research laboratory at the Murdoch Children's research institute is taking place. This is a WHO funded project on colonising pneumococci.

The Pneumococcal Serotype Replacement and Distribution Estimation (PSERENADE) Project: WHO also commissioned the Pneumococcal Serotype Replacement and Distribution Estimation (PSERENADE) project to summarize and estimate the impact of PCV10/13 programs on IPD incidence and serotype distribution among children and adults. Switzerland is actively collaborating and provides data which allow large scale metanalyses. Three publications have been released so far [9-11]

4. Transfer of results

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

The data collected in 2020 were sent to the FOPH on January 29, 2021.

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. Due to the COVID-19 pandemic the NZPn temporary faced some shortage of staff causing a delayed reporting for a few results in spring 2020.

5. Reporting

This report includes data of the NZPn from 2020. 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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11. 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, 26.06.2021

Prof. Dr. med. Stephen L. Leib

Dr. phil. nat. Carlo Casanova

PD Dr. phil. nat. Markus Hilty