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Annual Report of the National Center for invasive Pneumococci (NZPn), 2023

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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 Prof. 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 2023 the NZPn received 1'076 isolates recovered from IPD. Thereof, 29 isolates were not *S. pneumoniae* or could not be cultured after transport, even after re-submission. Of the *S. pneumoniae* isolates 17 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 1'030 isolates were included. Thereof, 914 strains were isolated from blood, 18 from cerebrospinal fluid, 10 from pleural fluid, 2 from synovial fluid and 86 from other or not declared sites. After a decrease during the COVID-19 pandemic in 2020 and 2021 the total annual number of IPD isolates in 2023 was even higher than in the years before the pandemic (Figure 1).
- 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.

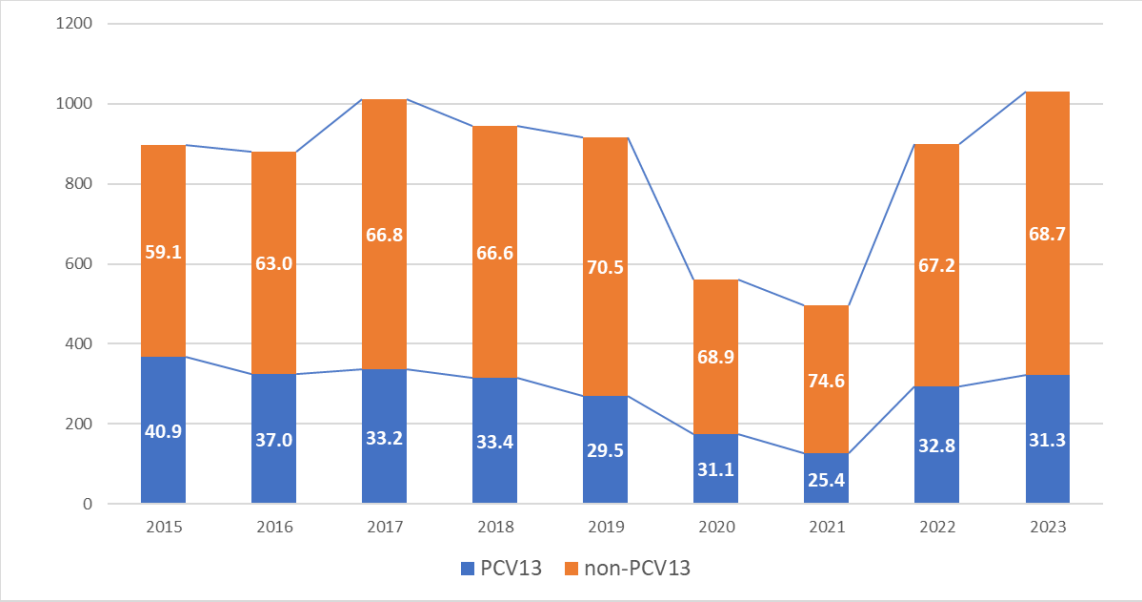


Figure 1 *Annual numbers of IPD isolates referred to the NZPn. The numbers in white indicate the proportion of non-PCV13 serotype isolates (%)*

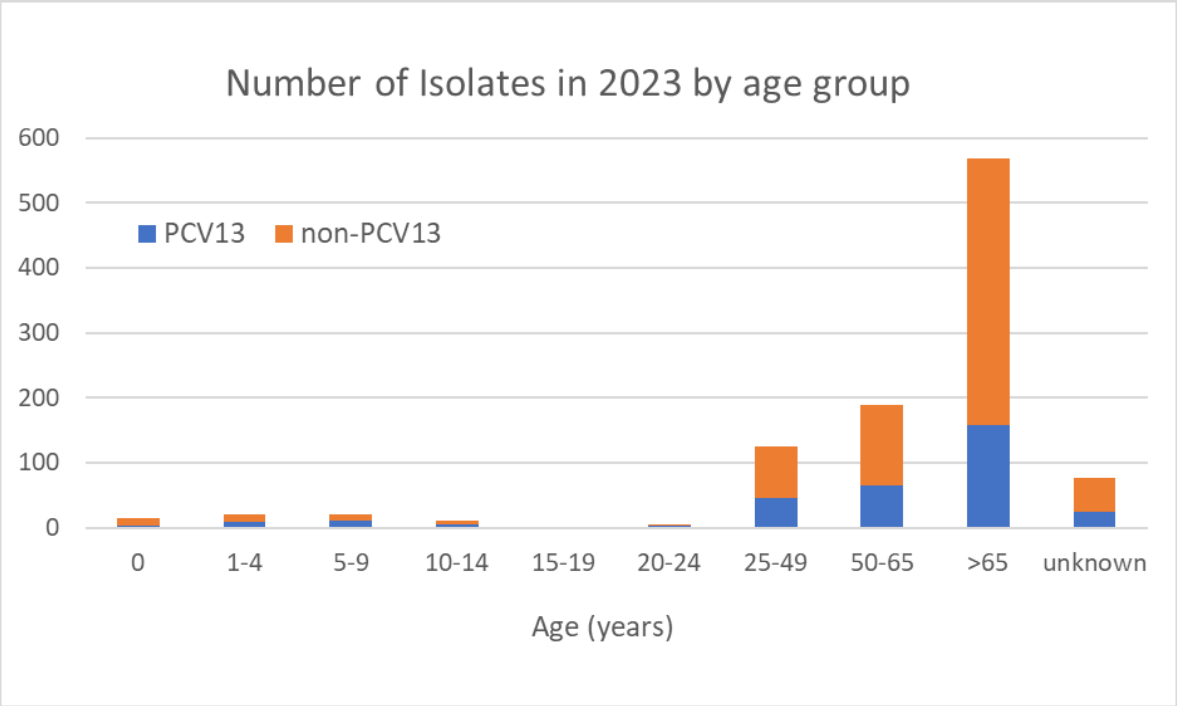


Figure 2 *Number of IPD isolates in 2023 by age group*

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 19'000 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. As previously experienced with PCV7, PCV13 induced a reduction of PCV13 serotypes but caused emergence of non-vaccine serotypes. Newer vaccines cover additional serotypes (PCV15 and PCV20).

- **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). Based on the actual serotype epidemiology, the NZPn evaluates at the beginning of the year if new or additional pneumococcal antisera have to be implemented in the diagnostic evaluation. In 2023, 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 2023, the NZPn has received 1030 non-duplicate strains of *Streptococcus pneumoniae* isolated from normally sterile body sites.

  The five most frequent serotypes in 2023 were serotype 8 (n=192), serotype 3 (n=189), serotype 22F (n=81), serotype 9N (n=60) and serotype 4 (n=46) (Table 2 and Figure 3), which, with exception of serotype 4, were the dominant serotypes already in the year before. Compared to 2022 there was a relative increase in serotype 4 and 14 IPD isolates (from 1.4 to 4.5% and 0.4 to 1.4%, respectively) and a decrease in serotype 19F isolates (from 4.1 to 2%). For the non-PCV13 isolates there were no major changes in the serotype distribution except for a slight increase in serotype 38 (from 0.4 to 1.4%). In 2023 only 31.3% of all IPD isolates were PCV13 serotypes. Newer vaccines cover following additional serotypes: 22F and 33F in PCV15; 8, 10A, 11A, 12F, 15B, 22F and 33F in PCV20. The proportion of these additional serotypes in 2023 were 9.2% for PCV15 (total coverage 40%) and 37.1% for PCV20 (total coverage 68%).

***Table 2**: Serotype distribution of referred IPD isolates 2019-2023. 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, 15B/C consisted of 15B (n=18) and15C (n=9) isolates, for one isolate the serotype (15B or C) could not be determined conclusively.*

	2019		2020		2021		2022		2023	
Serotype	n	%	n	%	n	%	n	%	n	%
3	**149**	**16.3**	**95**	**16.9**	**75**	**15.1**	**189**	**21.0**	**189**	**18.3**
19A	31	3.4	**25**	**4.4**	18	3.6	**37**	**4.1**	36	3.5
7F	6	0.7	4	0.7	1	0.2	3	0.3	2	0.2
19F	23	2.5	14	2.5	19	3.8	**37**	**4.1**	21	2.0
4	8	0.9	6	1.1	6	1.2	13	1.4	**46**	**4.5**
14	22	2.4	13	2.3	2	0.4	4	0.4	14	1.4
6A	4	0.4	4	0.7	0	0.0	1	0.1	1	0.1
9V	12	1.3	0	0.0	0	0.0	3	0.3	3	0.3
1	1	0.1	0	0.0	0	0.0	0	0.0	0	0.0
6B	4	0.4	5	0.9	1	0.2	3	0.3	3	0.3
18C	8	0.9	4	0.7	3	0.6	3	0.3	3	0.3
23F	2	0.2	5	0.9	1	0.2	2	0.2	4	0.4
5	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
Total PCV13	270	29.5	175	31.1	126	25.4	295	32.8	322	31.3
8	**142**	**15.5**	**96**	**17.1**	**114**	**23.0**	**194**	**21.6**	**192**	**18.6**
22F	**103**	**11.3**	**43**	**7.7**	**29**	**5.8**	**66**	**7.3**	**81**	**7.9**
9N	**63**	**6.9**	**33**	**5.9**	**29**	**5.8**	**47**	**5.2**	**60**	**5.8**
24*	13	1.4	12	2.1	8	1.6	23	2.6	25	2.4
15A	29	3.2	11	2.0	10	2.0	28	3.1	28	2.7
12F	**48**	**5.2**	13	2.3	7	1.4	13	1.4	10	1.0
10A	30	3.3	19	3.4	16	3.2	20	2.2	34	3.3
15B/C*	19	2.1	21	3.7	11	2.2	20	2.2	28	2.7
6C	13	1.4	10	1.8	8	1.6	10	1.1	17	1.7
11A	19	2.1	12	2.1	19	3.8	14	1.6	23	2.2
23B	23	2.5	18	3.2	**30**	**6.0**	29	3.2	28	2.7
23A	29	3.2	14	2.5	16	3.2	18	2.0	31	3.0
35F	20	2.2	12	2.1	12	2.4	23	2.6	17	1.7
31	7	0.8	6	1.1	4	0.8	6	0.7	13	1.3
38	11	1.2	5	0.9	1	0.2	4	0.4	14	1.4
16*	11	1.2	11	2.0	5	1.0	10	1.1	14	1.4
20	12	1.3	3	0.5	5	1.0	7	0.8	10	1.0
33F	13	1.4	11	2.0	9	1.8	11	1.2	14	1.4
17F	11	1.2	2	0.4	6	1.2	8	0.9	8	0.8
10B	3	0.3	4	0.7	7	1.4	5	0.6	11	1.1
35B	5	0.5	10	1.8	7	1.4	6	0.7	11	1.1
7	6	0.7	5	1.8	5	1.0	14	1.6	16	1.6
Other	15	1.6	16	3.7	12	2.4	29	3.2	23	2.2
Total non-PCV13	645	70.5	387	68.9	370	74.6	605	67.2	708	68.7
Total	**915**	100	**562**	100	**496**	100.0	**900**	100.0	**1030**	**100.0**

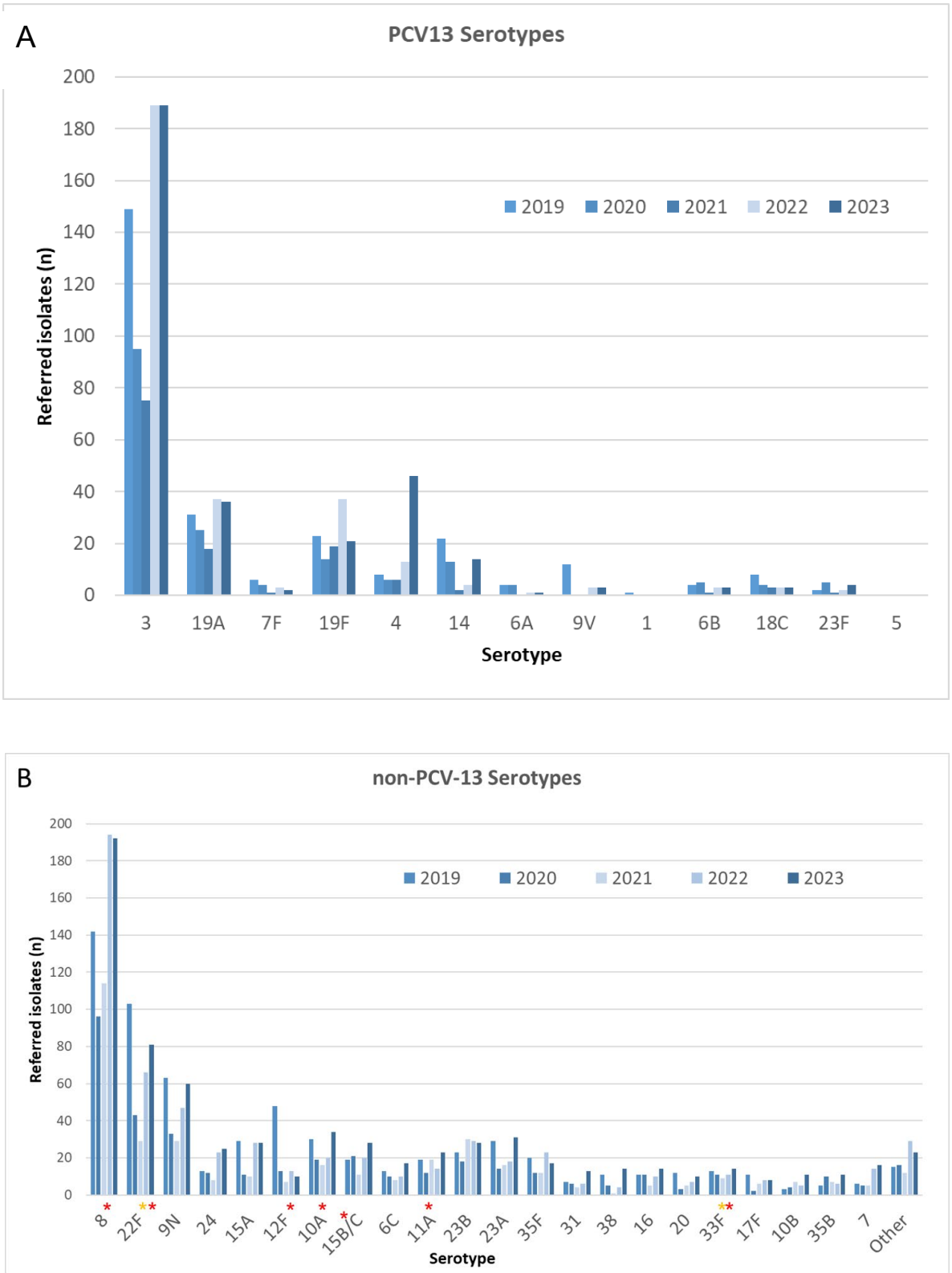


Figure 3 *Serotype distribution of invasive S. pneumoniae, annual absolute frequencies in 2019-2023 (A) PCV13 serotypes; (B) non-PCV13 serotypes. Additional non-PCV13 serotypes covered by the newer vaccines are indicated by asterisk: (*) PCV15, (*) PCV20*

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.

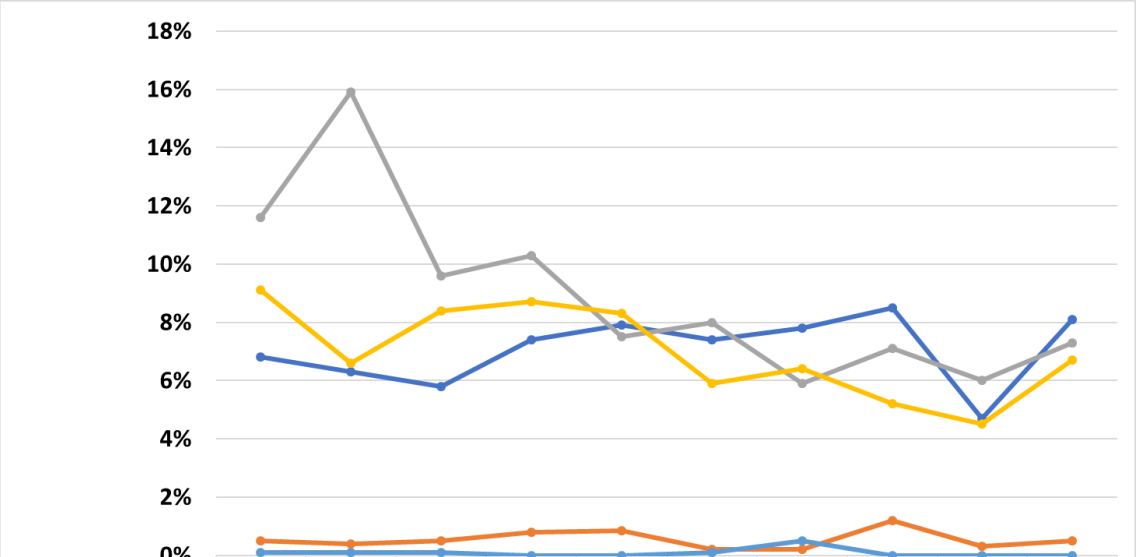
Table 3: Interpretive standards for *S. pneumoniae* according to EUCAST v13.0. DD, disc diffusion; MIC, minimal inhibitory concentration; S, susceptible; R, resistant

Antimicrobial agent		EUCAST 2023	
		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	<22
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_13.1_Breakpoint_Tables.pdf ** "Susceptible, increased exposure (I)", http://www.eucast.org/clinical_breakpoints/

Results: After a decrease in the proportion of IPD isolates with reduced penicillin susceptibility in 2022 (4.7% non-susceptible according to meningitis criteria) there was again an increase in 2023 (8.1%) to a similar rate as in the years before (7.8% in 2020, 8.5% in 2021). Thereof three isolates (0.3%) were

resistant by non-meningitis criteria (MIC > 2 µg/ml). Five isolates (0.5%) were non-susceptible to ceftriaxone (resistant by meningitis criteria), whereof one isolate was resistant also by non-meningitis criteria with an MIC of 3 µg/ml. 6.7% of the isolates were non-susceptible (intermediate or resistant) to erythromycin and 7.3% to trimethoprim-sulfamethoxazole. All isolates were susceptible (increased exposure/dose) to levofloxacin.
Compared to 2022, the resistance rate of IPD isolates in Switzerland thus increased in 2023 for all tested antibiotics except for levofloxacin.



	2014	2015	2016	2017	2018	2019	2020	2021	2022	2023
Penicillin	6.8%	6.3%	5.8%	7.4%	7.9%	7.4%	7.8%	8.5%	4.7%	8.1%
Ceftriaxone	0.5%	0.4%	0.5%	0.8%	0.8%	0.2%	0.2%	1.2%	0.3%	0.5%
Trim./Sulfa.	11.6%	15.9%	9.6%	10.3%	7.5%	8.0%	5.9%	7.1%	6.0%	7.3%
Erythromycin	9.1%	6.6%	8.4%	8.7%	8.3%	5.9%	6.4%	5.2%	4.5%	6.7%
Levofloxacin	0.1%	0.1%	0.1%	0.0%	0.0%	0.1%	0.5%	0.0%	0.0%	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 and ceftriaxone meningitis interpretive criteria were applied.*

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 2023.

2.1.6 Research in development of new diagnostic tools and antibacterial compounds

We assessed, the antibacterial activity of 22 thiolato-bridged dinuclear ruthenium(II)-arene compounds in vitro against *Escherichia coli*, *Streptococcus pneumoniae,* and *Staphylococcus aureus*. Some of the diruthenium(II)-arene compounds exhibited promising activity against *S. aureus* and *S. pneumoniae* but further studies are needed [1].

2.1.7 Epidemiological Research

The Invasive Respiratory Infection Surveillance (IRIS) Consortium was established to assess the impact of the COVID-19 pandemic on invasive diseases caused by *Streptococcus pneumoniae, Haemophilus influenzae*, *Neisseria meningitidis*, and *Streptococcus agalactiae* [2]. Switzerland is taking part in IRIS and sent data to the consortium. In total, data sets from laboratories in 30 countries are analysed. It was found that IPD cases began to increase in some countries towards the end of 2021 as the COVID-19 pandemic restrictions were lifted [2].

2.1.8 Additional pneumococcal research at the NZPn

In general and as shown above, serotype 8 is increasing in prevalence (Figure 3). Therefore, we tested the virulence of such an isolate in a rat model of meningitis in comparison with a serotype 15B and a serotype 14 isolate [3]. We found that only the serotype 8 isolate was hypervirulent causing brain injury and a high mortality rate. The importance of the rat model in studying experimental meningitis has been outlined by us in 2023 (DOI: 10.1016/B978-0-323-89833-1.00050-1).

The recommended empiric ceftriaxone dosing regimen for acute bacterial meningitis in adults is 2 g every 12 h [4]. In a study done in 2023, we found no statistical difference in outcome between the 2 g every 24 h and the 2 g every 12 h ceftriaxone dosing regimens. Our results indicate that a ceftriaxone total daily dose of 2 g may be associated with similar outcomes to a 4 g total daily dose, provided that the causative organism (e.g. *Streptococcus pneumoniae*) is highly susceptible to ceftriaxone.

We also investigated the influence of carbon sources on pneumococcal growth, capsule biosynthesis, and subsequent adherence potential [5]. Our findings suggested that a careful adaption of the lifestyle of *S. pneumoniae* according to the monosaccharides (e.g. Glucose and Galactose) encountered in the respective human niche [5]. In collaboration, we also investigated the composition of the nasopharyngeal and oropharyngeal microbiota of healthy adults, focusing on the effect of *Streptococcus pneumoniae* carriage [6]. Interestingly, this study showed that, in adults, the presence of *S. pneumoniae* in the nasopharynx is associated with a shift in the microbiota and dominance of the *Streptococcus* genus.

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 2019, 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 [7-9]. The collaboration has been ongoing in 2023 and more manuscripts are expected in 2024 and 2025.

IRIS network: As mentioned above. A new manuscript has been 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 and 2 publications will be published in 2024 and 2025

4. Transfer of results

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

The data collected in 2023 were sent to the FOPH on January 26, 2024.

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 2023.

5. Reporting

This report includes data of the NZPn from 2023. 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 (References)

1. Bugnon Q, Melendez C, Desiatkina O, Fayolles de Chaptes L, Holzer I, Paunescu E, Hilty M, Furrer J: **In vitro antibacterial activity of dinuclear thiolato-bridged ruthenium(II)-arene compounds.** *Microbiol Spectr* 2023, **11:**e0095423.
2. Shaw D, Abad R, Amin-Chowdhury Z, Bautista A, Bennett D, Broughton K, Cao B, Casanova C, Choi EH, Chu YW, et al: **Trends in invasive bacterial diseases during the first 2 years of the COVID-19 pandemic: analyses of prospective surveillance data from 30 countries and territories in the IRIS Consortium.** *Lancet Digit Health* 2023, **5:**e582-e593.
3. Muller A, Lekhuleni C, Hupp S, du Plessis M, Holivololona L, Babiychuk E, Leib SL, Grandgirard D, Iliev AI, von Gottberg A, Hathaway LJ: **Meningitis-associated pneumococcal serotype 8, ST 53, strain is hypervirulent in a rat model and has non-haemolytic pneumolysin which can be attenuated by liposomes.** *Front Cell Infect Microbiol* 2022, **12:**1106063.
4. Raemy S, Casanova C, Baldan R, Barreto E, Tande AJ, Endimiani A, Leib SL, Fischer U, Sendi P: **Penicillin-Susceptible *Streptococcus pneumoniae* Meningitis in Adults: Does the Ceftriaxone Dosing Matter?** *Antibiotics (Basel)* 2023, **12**.
5. Werren JP, Mostacci N, Gjuroski I, Holivololona L, Troxler LJ, Hathaway LJ, Furrer J, Hilty M: **Carbon source-dependent capsule thickness regulation in *Streptococcus pneumoniae*.** *Front Cell Infect Microbiol* 2023, **13:**1279119.
6. Paulo AC, Lanca J, Almeida ST, Hilty M, Sa-Leao R: **The upper respiratory tract microbiota of healthy adults is affected by *Streptococcus pneumoniae* carriage, smoking habits, and contact with children.** *Microbiome* 2023, **11:**199.
7. 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**.
8. 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**.
9. 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, 27.8.2024

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