Institute for Infectious Diseases, CH-3010 Bern

Institute for Infectious Diseases

Annual Report of the National Center for invasive Pneumococci (NZPn), 2018

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:

http://www.ifik.unibe.ch/dienstleistungen/pneumokokken_zentrum/centre_national_pour_les_pneumocoques_invasifs_mandat_par_lofsp/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

Table of contents

1. Organization 3
2. Diagnostics and quality assurance 3
2.1.1 Confirmatory diagnostics/national monitoring of quality 3
2.1.2 Strain collection 4
2.1.3 Serotyping of invasive pneumococcal isolates 4
2.1.4 Antibiotic resistance data of invasive pneumococcal isolates 8
2.1.5 National and International quality assurance 10
2.1.6 Research in development of new diagnostic tools 10
2.1.7 Epidemiological Research 11
2.1.8 Additional pneumococcal research at the NZPn 11
3. Advisory service and networking 12
3.1 Advisory service 12
3.2 Networking 12
4. Transfer of results 13
4.1 Transfer of data to the Federal Office of Public Health (FOPH) 13
4.2. Transfer of results to the referring laboratories 13
5. Reporting 13
6. Publications related to the topic within the reporting period (References) 13

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*. As for July 2016 onwards, the NZPn in its current form 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, DNA Probe analysis (AccuProbe *Streptococcus pneumoniae* culture identification test, Gen-Probe, Inc.)). 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). A DNA probe analysis and bile

solubility test is performed to differentiate these isolates from optochin susceptible non-pneumococcal viridans streptococci.

- **Results**: In 2018 the NZPn received 1'029 isolates recovered from IPD. Thereof 62 isolates were not *S. pneumoniae* or could not be cultured after transport. Of these, 10 isolates could be cultured after resubmission. Of the *S. pneumoniae* isolates 23 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 944 isolates of 941 patients were included (two patients had bacteremia with two different serotypes, one patient had two episodes with two different serotypes).

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 10'000 isolates. Biobanking of this large collection needs to be reorganized in the near future to comply with organizational standards. The FOPH will be invited to participate in the planning and support of such a biobank.

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 to be *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 2018, no additional adaptions have been made. 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	15	15A	15B/C	15F
16	17	17A	17F	18	18C	18F	19	19F	19A
20	21	22	22F	23	23A	23B	23F	24	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 2018, the NZPn has received 944 strains of *Streptococcus pneumoniae* isolated from normally sterile body sites. The number of isolates was lower than in the previous year (n=1’012), but higher than in 2016 (n=879) and 2015 (n=898) (Table 2). In total 44 different serotypes/serogroups were identified in 2018.

  As in the two years before the five most frequent serotypes were serotype 3 (n=187), serotype 8 (n=160), serotype 22F (n=86), serotype 9N (n=50) and serotype 19A (n=42), which together constituted more than 55% of the invasive isolates (Table 2 and Figure 1). The proportion of non-PCV13 serotype IPD isolates did not further increase in 2018 compared to the previous year (66.6% vs 66.8% in 2017; Table 2). The occurrence of the two most frequent serotypes 3 and 8 further increased in 2018 while other common PCV13 (19A, 7F, 19F, 4) and non-PCV13 (9N, 15A) serotypes decreased.

- **Conclusion**: In total there was no further shift from PCV13 to non-PCV13 serotypes in 2018 compared to the year before. Of note is, however, the persistent increase of the two most prevalent serotypes, the non-PCV13 serotype 8 and the PCV13 serotype 3. These two serotypes should certainly be a main focus in future vaccine design strategies.

Table 2: Serotype distribution of referred IPD isolates 2015-2018. The five most frequent serotypes in each year are indicated in bold numbers.

	2015		2016		2017		2018	
Serotype	n	%	n	%	n	%	n	%
3	**154**	**17.1**	**165**	**18.8**	**158**	**15.6**	**187**	**19.8**
19A	**67**	**7.5**	**46**	**5.2**	**63**	**6.2**	**42**	**4.4**
7F	**37**	**4.1**	26	3.0	22	2.2	9	1.0
19F	17	1.9	20	2.3	27	2.7	18	1.9
4	19	2.1	19	2.2	21	2.1	13	1.4
14	30	3.3	18	2.0	17	1.7	19	2.0
6A	11	1.2	7	0.8	5	0.5	4	0.4
9V	7	0.8	7	0.8	6	0.6	8	0.8
1	6	0.7	5	0.6	3	0.3	0	0.0
6B	4	0.4	5	0.6	9	0.9	4	0.4
18C	4	0.4	4	0.5	1	0.1	6	0.6
23F	10	1.1	3	0.3	4	0.4	5	0.5
5	1	0.1	0	0.0	0	0.0	0	0.0
Total PCV13	367	40.9	325	37.0	336	33.2	315	33.4
8	**89**	**9.9**	**107**	**12.2**	**150**	**14.8**	**160**	**16.9**
22F	**69**	**7.7**	**83**	**9.4**	**91**	**9.0**	**86**	**9.1**
9N	33	3.7	**56**	**6.4**	**64**	**6.3**	**50**	**5.3**
24	41	4.6	31	3.5	15	1.5	13	1.4
15A	21	2.3	26	3.0	31	3.1	27	2.9
12F	21	2.3	24	2.7	29	2.9	37	3.9
10A	21	2.3	22	2.5	24	2.4	25	2.6
15B/C	18	2.0	22	2.5	24	2.4	25	2.6
6C	26	2.9	21	2.4	22	2.2	15	1.6
11A	22	2.4	20	2.3	27	2.7	24	2.5
23B	30	3.3	20	2.3	39	3.9	33	3.5
23A	20	2.2	16	1.8	27	2.7	20	2.1
35F	20	2.2	16	1.8	15	1.5	16	1.7
31	12	1.3	12	1.4	18	1.8	6	0.6
38	10	1.1	12	1.4	5	0.5	6	0.6
16	6	0.7	11	1.3	15	1.5	9	1.0
20	21	2.3	11	1.3	17	1.7	13	1.4
33F	13	1.4	11	1.3	16	1.6	16	1.7
17F	8	0.9	8	0.9	8	0.8	8	0.8
10B	7	0.8	4	0.5	4	0.4	4	0.4
35B	9	1.0	3	0.3	14	1.4	11	1.2
Other	14	1.6	18	2.0	21	2.1	25	2.6
Total non-PCV13	531	59.1	554	63.0	676	66.8	629	66.6
Total	898	100	879	100	1012	100	944	100

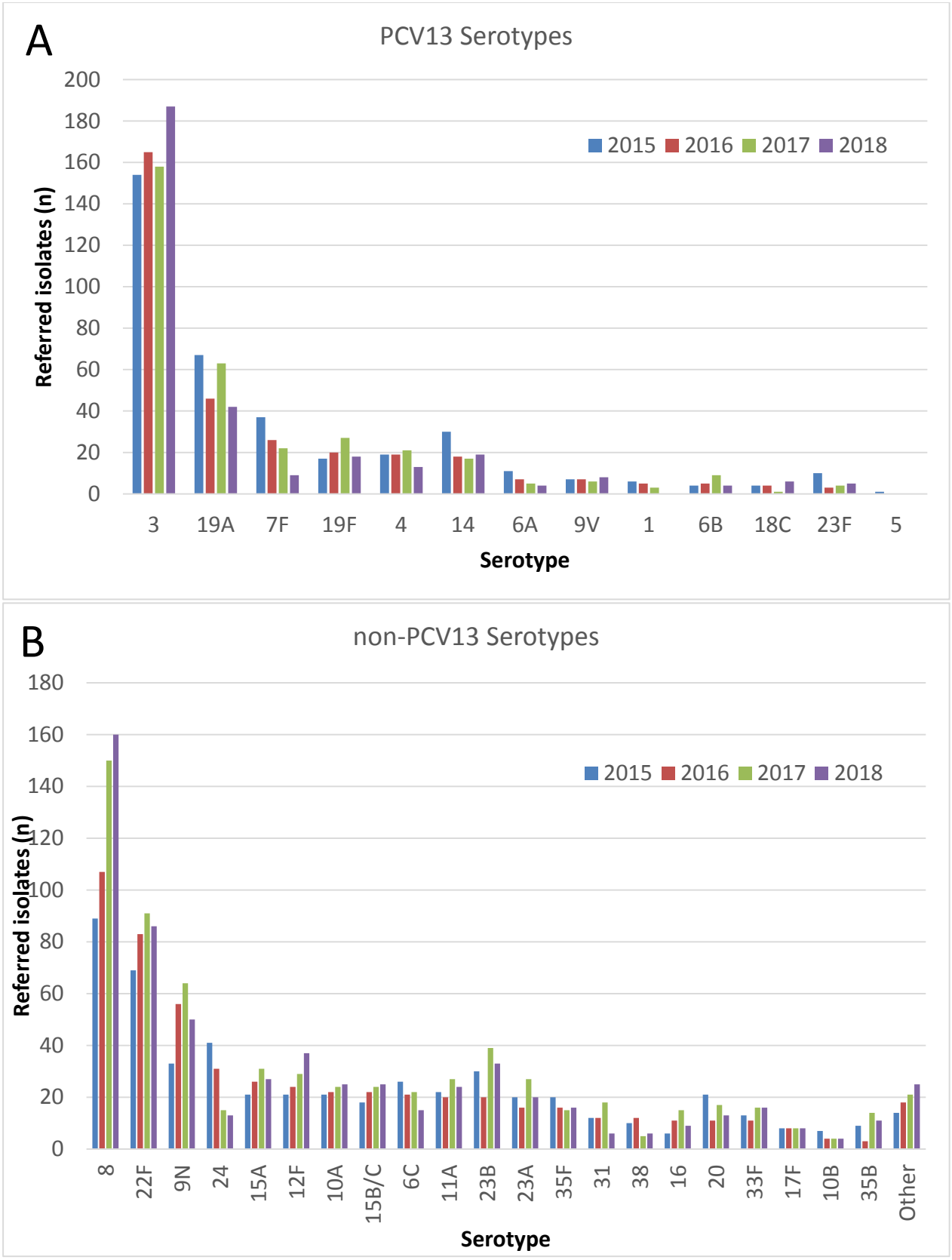


Figure 1: Serotype distribution of invasive *S. pneumoniae,* annual absolute frequencies in 2015-2018 (A) PCV13 serotypes; (B) non-PCV13 serotypes.

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 E-tests (bioMérieux, France and Liofilchem, Italy). Values of the E-test 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).

In order to monitor long-term antibiotic resistance trends, the NZPn kept using the American Clinical and Laboratory Standards Institute (CLSI) guidelines until December 2017. To provide a better comparability with susceptibility data of the referring diagnostic laboratories and other European countries we decided, however, to switch to EUCAST guidelines, starting from January 2018. This includes the use of a different culture medium (MH-F) and several differences in the interpretive criteria (indicated in bold in Table 3). In order to evaluate the influence of this change on susceptibility data we tested the referred isolates in parallel according to both guidelines. Comparison of the data for 590 isolates (January – June 2017) revealed a categoric agreement (susceptible/intermediate/resistant) of 99.1%. Applying the categories susceptible vs. non-susceptible (combining the intermediate and resistant categories) the agreement was 99.8%. We thus concluded that the susceptibility data generated using EUCAST guidelines will largely be comparable with our previous data, allowing a continuous surveillance.

- **Results:** In 2018, 75 isolates (7.9%) were resistant to penicillin using meningitis criteria (MIC >0.06 µg/mL). Compared to the previous years the proportion of penicillin non-susceptible isolates was thus again slightly increasing (6.3% in 2015, 5.8% in 2016, 7.4% in 2017; Figure 3). We did, however, not receive any isolate resistant by non-meningitis criteria (MIC >2 µg/ml), with the exception of one single isolate with an extraordinary high MIC of 8 µg/mL. This highly resistant isolate was in addition resistant to ceftriaxone (MIC 4 µg/mL), erythromycin and trimethoprim-sulfamethoxazole. Further seven isolates had a ceftriaxone MIC of 0.75 µg/mL (intermediate). Thus, in total 0.8% of the referred isolates were non-susceptible to ceftriaxone, which is the same rate as in 2017. For trimethoprim-sulfamethoxazole, 71 isolates revealed non-susceptibility (7.5 % versus 10.3% in 2017) while for erythromycin this was the case for 78 isolates (8.3% versus 8.7% in 2017). As in previous years, all isolates were susceptible to levofloxacin.

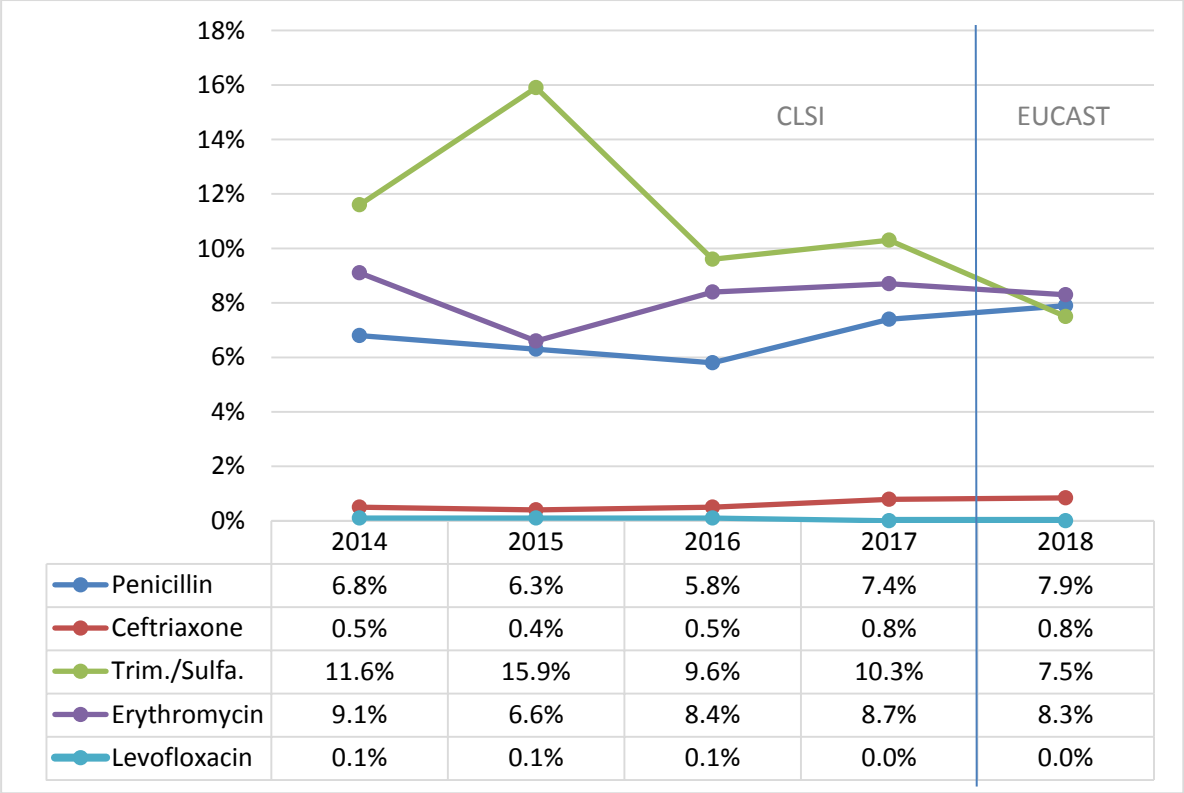
Table 3: Interpretive standards for *S. pneumoniae* according to CLSI 2018 and EUCAST v8.0. DD, disc diffusion; MIC, minimal inhibitory concentration; S, susceptible; R, resistant

Antimicrobial agent		CLSI		EUCAST	
		S	R	S	R
Penicilllin (oxacillin screen)	DD 1 μg oxacillin disc (mm)	≥20		≥20	
Penicillin parenteral (meningitis)	MIC (μg/ml)	≤0.06	**≥0.12**	≤0.06	**>0.06**
Penicillin parenteral (non-meningitis)	MIC (μg/ml)	**≤2**	**≥8**	**≤0.06***	**>2**
Ceftriaxone parenteral (meningitis)	MIC (μg/ml)	≤0.5	**≥2**	≤0.5	**>2**
Ceftriaxone parenteral (non-meningitis)	MIC (μg/ml)	**≤1**	**≥4**	**≤0.5**	**>2**
Trimethoprim-sulfamethoxazole	DD 1.25/ 23.75 μg disc (mm)	**≥19**	**≤15**	**≥18****	**<15****
Erythromycin	DD 15μg disc (mm)	**≥21**	**≤15**	**≥22**	**<19**
Levofloxacin	DD 5μg (mm)	≥17	**≤13**	**≥16**	**<16**

* Interpretation and dosing for non-meningitis isolates according to http://www.eucast.org/clinical_breakpoints/

** EUCAST v9.0 (2019): S ≥ 13; R <10mm

- **Conclusion:** Compared to 2017 the resistance rate of IPD isolates in Switzerland remained rather constant for most antibiotics tested. There was again a very slight increase in the penicillin resistance rate and we received one multidrug-resistant isolate with exceptionally high MICs for penicillin and ceftriaxone.
  Based on our internal evaluation we expect the change from CLSI to EUCAST standards to have only a minor influence on the susceptibility data. For trimethoprim-sulfamethoxazole, however, the decrease in resistance could, at least in part, be artificially caused by the application of the less stringent EUCAST interpretive criteria for susceptibility (Table 3 and Figure 3).



	2014	2015	2016	2017	2018
Penicillin	6.8%	6.3%	5.8%	7.4%	7.9%
Ceftriaxone	0.5%	0.4%	0.5%	0.8%	0.8%
Trim./Sulfa.	11.6%	15.9%	9.6%	10.3%	7.5%
Erythromycin	9.1%	6.6%	8.4%	8.7%	8.3%
Levofloxacin	0.1%	0.1%	0.1%	0.0%	0.0%

Figure 3: Proportion of non-susceptible IPD isolates (% intermediate or resistant). For penicillin the meningitis interpretive criteria were applied.

2.1.5 National and International quality assurance

In November 2018 the NZPn successfully participated in the international External Quality assurance (EQA) program organized by IBD-labnet / ECDC in collaboration with UK NEQAS. The schedule included *S. pneumoniae* strain identification/characterization (species identification, serotyping, MLST subtyping), antimicrobial susceptibility testing and non-culture detection of bacteria in simulated CSF samples from suspected meningitis cases. The results of the ring trial will be published by the ECDC.

2.1.6 Research in development of new diagnostic tools

- **Evaluation of different Nuclear Magnetic Resonance (NMR) methods for metabolomic studies in *Streptococcus pneumoniae*" (Poster presentation during SSM 2018, Lausanne;** https://www.swissmicrobiology.ch/events/annual-meeting-and-assembly/2018**).** Growth environment has been shown to influence thickness of the capsular polysaccharide in *Streptococcus pneumoniae*, but the mechanism of this influence is not precisely known. Several pneumococcal strains were cultured under various growth conditions and whole cell extracts of the bacteria were then analyzed using several

different NMR methods. Conclusion: Our findings will allow us to establish a detailed protocol for the determination of differences in CPS metabolism in different serotypes and with different carbon sources.

2.1.7 Epidemiological Research

- **Influence of older age and other risk factors on hospitalization for pneumococcal pneumoniae and detrimental outcome in adults (Poster presentation during ISPPD 2018, Melbourne;** https://isppd.kenes.com/2018/scientific-program-(2)/scientific-program#.XRxG4OszbmE**).** We used a database of all hospitalisations in Switzerland which includes ICD-10 diagnoses to obtain the number of hospitalisations for pneumococcal pneumonia among adults from 2002 to 2015. We calculated the effects of age and of those comorbidities, which are national 13-valent pneumococcal conjugate vaccine (PCV13) indications, on pneumococcal pneumonia hospitalisation, associated LOS and all-cause in-hospital mortality. Conclusion: Older age is a risk factor for hospitalisation with pneumococcal pneumonia and for longer LOS and higher mortality similarly to and independent of PCV13 indications
- **Serotype epidemiology of invasive Streptococcus pneumoniae in Switzerland, 2010-2017 (Poster presentation during SSM 2018, Lausanne;** https://www.swissmicrobiology.ch/events/annual-meeting-and-assembly/2018**).** We aimed at describing the serotype epidemiology of invasive pneumococcal disease (IPD) in Switzerland from 2010 to 2017, i.e. since the introduction of the seven-valent (PCV7) (in 2007) and the thirteen-valent pneumococcal conjugate vaccine PCV13 (in 2011). Conclusion: There is evidence that the introduction of PCV7 and PCV13 in Switzerland has contributed to a significant decline of PCV7 and five of the additional six PCV13 serotypes regarding invasive pneumococcal disease from 2010 to 2017.

2.1.8 Additional pneumococcal research at the NZPn

We were co-authoring a study which identified 13 core molecules associated with seven serotypes (6B, 14, 15, 18C, 19F, 9V, and 23F), and seven molecules that were differentially produced between serotypes [1]. We also investigated ere, the ability of the three oligopeptide-binding proteins AmiA, AliA, and AliB of an ATP-binding cassette transporter of pneumococcus to detect short peptides found in other bacterial species [2]. We also revealed how Foreign peptide triggers boost in pneumococcal metabolism and growth [3] and that peptide Ligands of AmiA, AliA, and AliB Proteins Determine Pneumococcal Phenotype [4]. We also investigated the combined effect of non-bacteriolytic antibiotic and inhibition of matrix metalloproteinases prevents brain injury and preserves learning, memory and hearing function in experimental paediatric pneumococcal meningitis [5]. We found

that combined adjuvant therapy with the non-bacteriolytic antibiotic daptomycin and the MMP inhibitor Trocade integrates the neuroprotective effects of both single adjuvants in experimental paediatric pneumococcal meningitis by reducing neuroinflammation and brain damage, thereby improving neurofunctional outcome [5]. We have summarized novel and preclinical treatment strategies in pneumococcal meningitis in a recent review [6]. All the studies are mentioned in the References section below.

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 [7]. 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 collaborate with Mark van der Linden for a project to identify the following, potentially new serotypes: 6D, 6E, 6F and 6G. We share isolates and aim at characterizing using NMR and whole genome sequencing methods.

ISPPD, Melbourne, Australia (https://isppd.kenes.com/2018/): We actively took part at the 11th International Symposium on Pneumococci and Pneumococcal Diseases (ISPPD 2018) which took place in Melbourne, Australia (Member of the faculty, oral and poster presentation). Networking took place among the key opinion leaders of pneumococcal research.

University of the Witwatersrand, South Africa: We have a joint project including our institution and several universities of South Africa, including the University of the Witwatersrand. 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.

4. Transfer of results

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

The data collected in 2018 were sent to the FOPH on February 25, 2019.

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 2018.

5. Reporting

This report includes data of the NZPn from 2018. 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. Mellors TR, Rees CA, Franchina FA, Burklund A, Patel C, Hathaway LJ, Hill JE: **The volatile molecular profiles of seven Streptococcus pneumoniae serotypes.** *J Chromatogr B Analyt Technol Biomed Life Sci* 2018, **1096:**208-213.
2. Nasher F, Heller M, Hathaway LJ: **Streptococcus pneumoniae Proteins AmiA, AliA, and AliB Bind Peptides Found in Ribosomal Proteins of Other Bacterial Species.** *Front Microbiol* 2017, **8:**2688.
3. Nasher F, Forster S, Yildirim EC, Grandgirard D, Leib SL, Heller M, Hathaway LJ: **Foreign peptide triggers boost in pneumococcal metabolism and growth.** *BMC Microbiol* 2018, **18:**23.
4. Nasher F, Aguilar F, Aebi S, Hermans PWM, Heller M, Hathaway LJ: **Peptide Ligands of AmiA, AliA, and AliB Proteins Determine Pneumococcal Phenotype.** *Front Microbiol* 2018, **9:**3013.
5. Muri L, Grandgirard D, Buri M, Perny M, Leib SL: **Combined effect of non-bacteriolytic antibiotic and inhibition of matrix metalloproteinases prevents brain injury and preserves learning, memory and hearing function in experimental paediatric pneumococcal meningitis.** *J Neuroinflammation* 2018, **15:**233.
6. Bewersdorf JP, Grandgirard D, Koedel U, Leib SL: **Novel and preclinical treatment strategies in pneumococcal meningitis.** *Curr Opin Infect Dis* 2018, **31:**85-92.
7. Pai R, Gertz RE, Beall B: **Sequential multiplex PCR approach for determining capsular serotypes of Streptococcus pneumoniae isolates.** *J Clin Microbiol* 2006, **44:**124-131.

Bern, 11.07.2019

Prof. Dr. med. Stephen L. Leib

Dr. phil. nat. Carlo Casanova

PD Dr. phil. nat. Markus Hilty