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Annual Report of the National Center for invasive Pneumococci (NZPn), 2019

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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*. 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
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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 are performed to differentiate these isolates from optochin susceptible non-pneumococcal viridans streptococci.

- **Results**: In 2019 the NZPn received 960 isolates recovered from IPD. Thereof, 30 isolates were not *S. pneumoniae* or could not be cultured after transport. Of the *S. pneumoniae* isolates 15 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 915 isolates of 908 patients were included (four patients had two episodes with two different serotypes, two patients had two episodes with the same serotype and one patient had one episode 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 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 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 2019, we introduced additional factor antisera to have a better resolution within serogroups 15, 16 and 24. 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 2019, the NZPn has received 915 non-duplicate strains of *Streptococcus pneumoniae* isolated from normally sterile body sites. The number of isolates was lower than in the previous two years (n=1'012 and n=944 in 2017 and 2018), but higher than in 2015 (n=898) and 2016 (n=879) (Table 2). In total 46 different serotypes/serogroups were identified in 2019.

  The five most frequent serotypes in 2019 were serotype 3 (n=149), serotype 8 (n=142), serotype 22F (n=103), serotype 9N (n=63) and serotype 12F (n=48) (Table 2 and Figure 1). Although there was a slight decrease in serotype 3 and serotype 8 isolates, these two serotypes are still responsible for a large proportion of IPD cases (16.3% and 15.5%, respectively). In 2019 there was again an increase in the proportion of non-PCV13 serotype IPD isolates (70.5% vs 66.8% and 66.6% in 2017 and 2018, respectively; Table 2). Responsible for this "serotype-replacement" is, on one hand, the ongoing decrease in PCV13 isolates. In particular, serotype 19A (in contrast to serotype 19F) and serotype 7F (which used to be among the top-five serotypes five years ago) were again decreasing in 2019. On the other hand, non-vaccine serotypes, such as 22F (11.3%), 9N (6.9%), 12F (5.2%) or 10A (3.3%), continued to increase.
  The introduction of the new factor sera revealed the presence of both 15B (n=11) and 15C (n=7) serotypes (plus one inconclusive B/C), whereas serogroup 16 and 24 exclusively consisted of 16F and 24F serogroup isolates.

  Conclusion: While the shift from PCV13 to non-PCV13 serotypes halted between 2017 and 2018, this trend continued in 2019. Only 29.5% of IPD isolates were PCV13 serotypes, whereas emerging non-vaccine serotypes represented now 70.5%. With the increase of serotype 12F, four of the five most frequent serotypes were, for the first time, non-PCV13 types.

Table 2: Serotype distribution of referred IPD isolates 2015-2019. The five most frequent serotypes in each year are indicated in red.

	2015		2016		2017		2018		2019	
Serotype	n	%	n	%	n	%	n	%	n	%
3	**154**	**17.1**	**165**	**18.8**	**158**	**15.6**	**187**	**19.8**	**149**	**16.3**
19A	**67**	**7.5**	**46**	**5.2**	**63**	**6.2**	**42**	**4.4**	31	3.4
7F	**37**	**4.1**	26	3.0	22	2.2	9	1.0	6	0.7
19F	17	1.9	20	2.3	27	2.7	18	1.9	23	2.5
4	19	2.1	19	2.2	21	2.1	13	1.4	8	0.9
14	30	3.3	18	2.0	17	1.7	19	2.0	22	2.4
6A	11	1.2	7	0.8	5	0.5	4	0.4	4	0.4
9V	7	0.8	7	0.8	6	0.6	8	0.8	12	1.3
1	6	0.7	5	0.6	3	0.3	0	0.0	1	0.1
6B	4	0.4	5	0.6	9	0.9	4	0.4	4	0.4
18C	4	0.4	4	0.5	1	0.1	6	0.6	8	0.9
23F	10	1.1	3	0.3	4	0.4	5	0.5	2	0.2
5	1	0.1	0	0.0	0	0.0	0	0.0	0	0.0
Total PCV13	367	40.9	325	37.0	336	33.2	315	33.4	270	29.5
8	**89**	**9.9**	**107**	**12.2**	**150**	**14.8**	**160**	**16.9**	**142**	**15.5**
22F	**69**	**7.7**	**83**	**9.4**	**91**	**9.0**	**86**	**9.1**	**103**	**11.3**
9N	33	3.7	**56**	**6.4**	**64**	**6.3**	**50**	**5.3**	**63**	**6.9**
24	41	4.6	31	3.5	15	1.5	13	1.4	13	1.4
15A	21	2.3	26	3.0	31	3.1	27	2.9	29	3.2
12F	21	2.3	24	2.7	29	2.9	37	3.9	**48**	**5.2**
10A	21	2.3	22	2.5	24	2.4	25	2.6	30	3.3
15B/C	18	2.0	22	2.5	24	2.4	25	2.6	19	2.1
6C	26	2.9	21	2.4	22	2.2	15	1.6	13	1.4
11A	22	2.4	20	2.3	27	2.7	24	2.5	19	2.1
23B	30	3.3	20	2.3	39	3.9	33	3.5	23	2.5
23A	20	2.2	16	1.8	27	2.7	20	2.1	29	3.2
35F	20	2.2	16	1.8	15	1.5	16	1.7	20	2.2
31	12	1.3	12	1.4	18	1.8	6	0.6	7	0.8
38	10	1.1	12	1.4	5	0.5	6	0.6	11	1.2
16	6	0.7	11	1.3	15	1.5	9	1.0	11	1.2
20	21	2.3	11	1.3	17	1.7	13	1.4	12	1.3
33F	13	1.4	11	1.3	16	1.6	16	1.7	13	1.4
17F	8	0.9	8	0.9	8	0.8	8	0.8	11	1.2
10B	7	0.8	4	0.5	4	0.4	4	0.4	3	0.3
35B	9	1.0	3	0.3	14	1.4	11	1.2	5	0.5
Other	14	1.6	18	2.0	21	2.1	25	2.6	21	2.3
Total non-PCV13	531	59.1	554	63.0	676	66.8	629	66.6	645	70.5
Total	**898**	100	**879**	100	**1012**	100	**944**	100	**915**	100

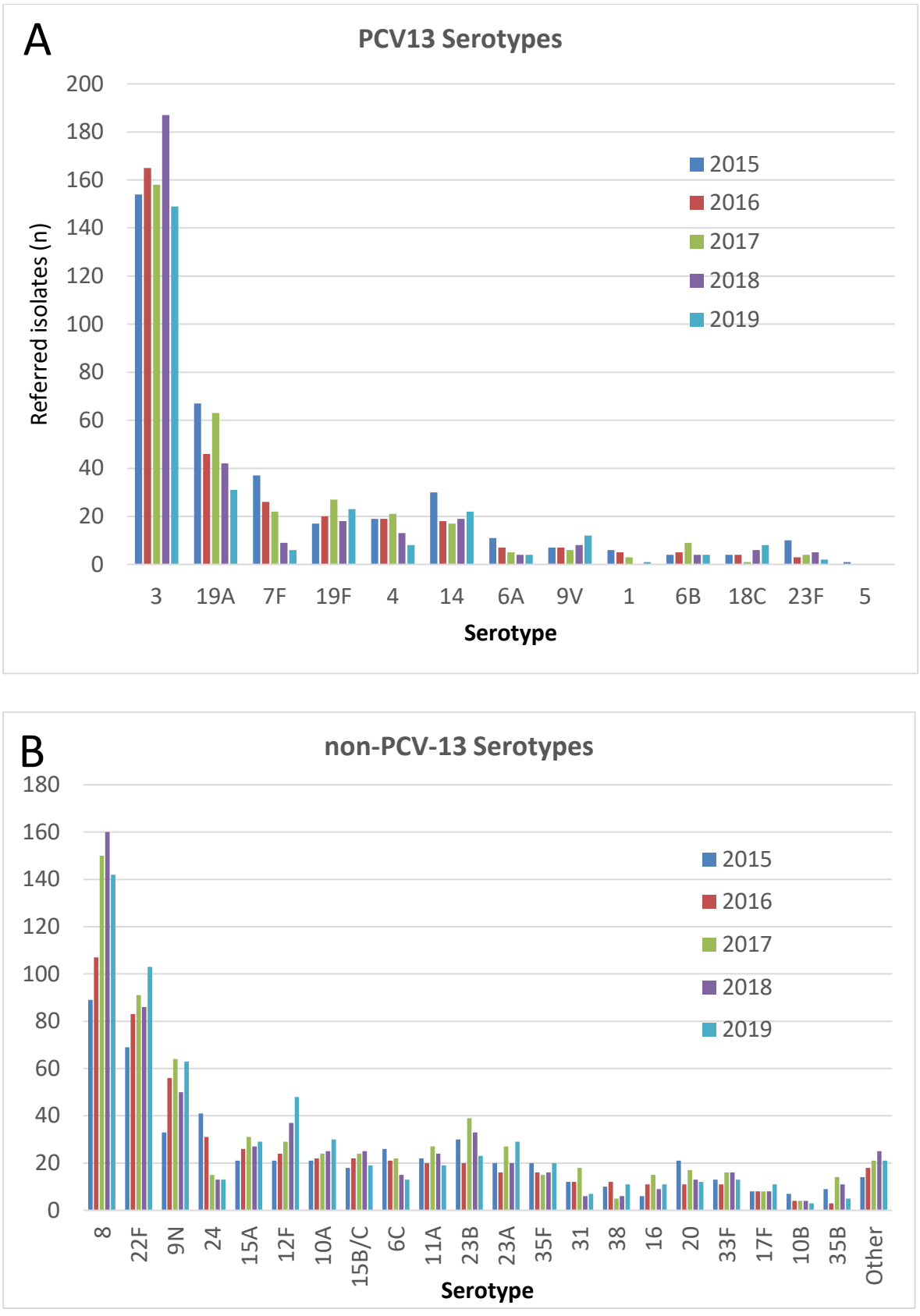


Figure 1: Serotype distribution of invasive *S. pneumoniae,* annual absolute frequencies in 2015-2019 (A) PCV13 serotypes; (B) non-PCV13 serotypes.

"Other" serotypes/serogroups included 7 (n=6), 13 (n=1), 15F (n=1), 18 (n=4), 18F (n=1), 21 (n=1), 22 (n=2), 27 (n=1), 28 (n=2), 34 (n=1) and 37 (n=1)

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.

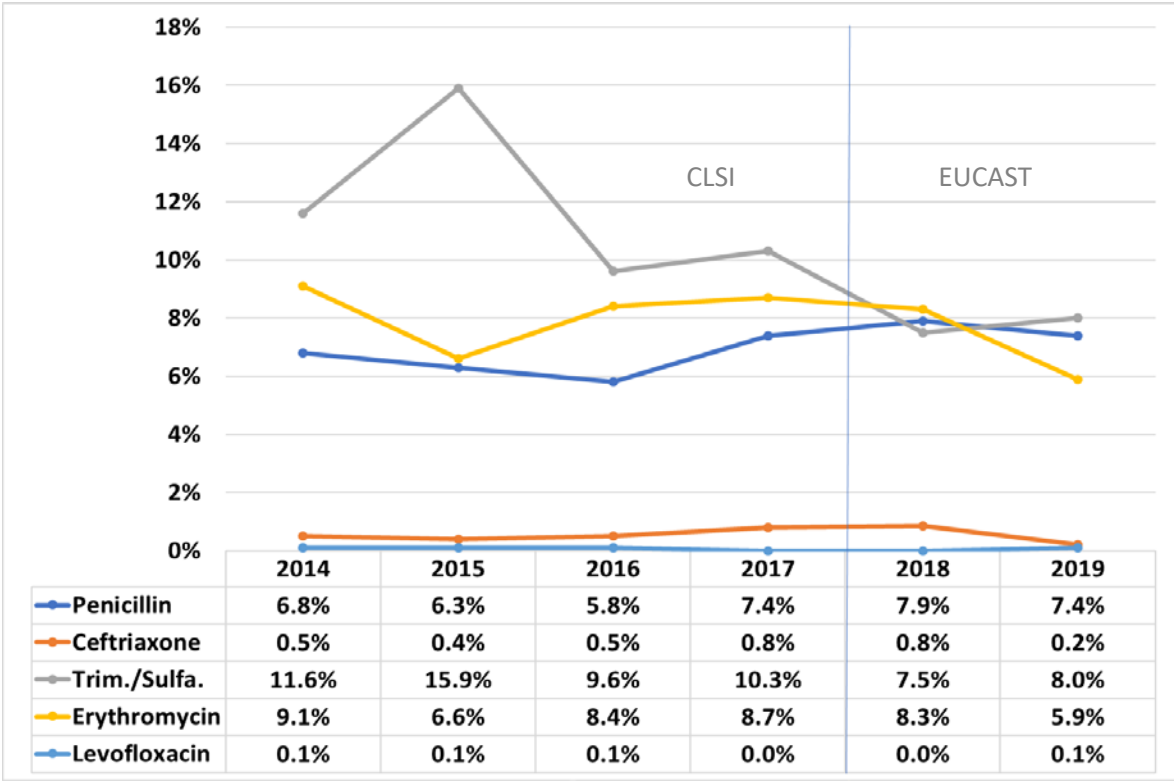
Table 3: Interpretive standards for *S. pneumoniae* according to CLSI 2019 and EUCAST v9.0. DD, disc diffusion; MIC, minimal inhibitory concentration; S, susceptible; R, resistant

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	**≥13****	**<10****
Erythromycin	DD 15µg disc (mm)	≥21	≤15	≥22	<19
Levofloxacin	DD 5µg (mm)	≥17	≤13	≥16*	<16

* Interpretation and dosing according to http://www.eucast.org/clinical_breakpoints/

** New breakpoints (EUCAST v8.0 (2018): S ≥ 18; R <15mm)

- **Results:** In 2019, 68 isolates (7.4%) 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 2017, 7.9% in 2018; Figure 3). All isolates were susceptible by non-meningitis criteria (MIC ≤ 2 µg/ml) and only two isolates were non-susceptible to ceftriaxone (MIC 0.75 = intermediate; 0.2%). 5.9% of the isolates were non-susceptible (intermediate or resistant) to erythromycin and 8% to trimethoprim-sulfamethoxazole. One isolate was resistant to levofloxacin (0.1%).



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

Figure 3: Proportion of non-susceptible IPD isolates (% intermediate or resistant). 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 2018 non-susceptibility slightly increased for trimethoprim-sulfamethoxazole (from 7.5% to 8%) and decreased for penicillin (from 7.9% to 7.4%), ceftriaxone (from 0.8% to 0.2%) and erythromycin (from 8.3% to 5.9%).

2.1.5 National and International quality assurance

No international quality assurance was carried out in 2019. The next External Quality assurance (EQA) program organized by IBD-labnet / UK NEQAS is scheduled for 2020.

2.1.6 Research in development of new diagnostic tools

- **Carbon Source Regulates Polysaccharide Capsule Biosynthesis in *S. pneumoniae* (Published in Journal of Biological Chemistry).** Growth environment has been shown to influence thickness of the capsular polysaccharide in *S. pneumoniae*, but the mechanism of this influence has not been 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: We revealed the mechanisms behind capsule thickness differences for growth media containing different carbon sources. Using the appropriate growth media may help for the identification of *S. pneumoniae* including serotype characterization. This study has been published in 2019 and is mentioned in the References section below [1].

2.1.7 Epidemiological Research

- **The Association Between Pneumococcal Vaccination, Ethnicity, and the Nasopharyngeal Microbiota of Children in Fiji. (Publication in 'Microbiome').** In collaboration with our Australian collegues, we examined the nasopharyngeal microbiota of 132 Fijian children using nasopharyngeal swabs collected from 12-month-old iTaukei and FID children who were vaccinated (3 doses PCV7) or unvaccinated in infancy as part of a phase II randomised controlled trial. We found that associations between PCV7 and nasopharyngeal microbiota differed within each ethnic group. This study highlights the influence that ethnicity and upper respiratory tract infections have on nasopharyngeal microbiota. This study has been published in 2019 and is mentioned in the References section below [2].

2.1.8 Additional pneumococcal research at the NZPn

We were co-authoring a study which studied the effect of pneumococcal factors, including the cholesterol-dependent cytolysin pneumolysin and the pneumococcal capsule, on microglial motility and taxis [3]. We also investigated the effect of binding of AliB-like ORF 1 peptide on the transcriptome and proteome of non-encapsulated pneumococci [4]. Furthermore, we analyzed the effect of local and systemic delivery of HspB5 in an infant rat model of pneumococcal meningitis [5]. We also aimed to improve the outcome of experimental pneumococcal meningitis by simultaneously targeting different pathophysiological mechanisms with combined adjunctive therapies previously shown to be neuroprotective. Within the study, we identified a triple-antibiotic therapy as a promising therapeutic option for pediatric pneumococcal meningitis [6]. Finally, we evaluated the effect of metformin adjunctive to antibiotics on neuroinflammation, brain and inner ear

damage, and neurofunctional outcome in experimental pediatric pneumococcal meningitis. The results identified adjuvant metformin as a promising therapeutic option to improve the outcome after pediatric pneumococcal meningitis [7].

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 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.

Europneumo 2019 in Greifswald, Germany : We actively took part at the European meeting for Pneumococci and presented our data (poster presentation). Networking took place among the European 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.

Murdoch Childrens Research Institute, Australia: A research collaboration with the head of the microbiological research laboratory at the Murdoch Childrens research institute is taking place. A collaborative project has been recently published (see above) [2].

4. Transfer of results

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

The data collected in 2019 were sent to the FOPH on February 23, 2020.

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 2019.

5. Reporting

This report includes data of the NZPn from 2019. 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. Troxler LJ, Werren JP, Schaffner TO, Mostacci N, Vermathen P, Vermathen M, Wuthrich D, Simillion C, Brugger SD, Bruggmann R, et al: **Carbon source regulates polysaccharide capsule biosynthesis in *Streptococcus pneumoniae*.** *J Biol Chem* 2019, **294:**17224-17238.
2. Boelsen LK, Dunne EM, Mika M, Eggers S, Nguyen CD, Ratu FT, Russell FM, Mulholland EK, Hilty M, Satzke C: **The association between pneumococcal vaccination, ethnicity, and the nasopharyngeal microbiota of children in Fiji.** *Microbiome* 2019, **7:**106.
3. Hupp S, Grandgirard D, Mitchell TJ, Leib SL, Hathaway LJ, Iliev AI: **Pneumolysin and the bacterial capsule of *Streptococcus pneumoniae* cooperatively inhibit taxis and motility of microglia.** *J Neuroinflammation* 2019, **16:**105.
4. Nasher F, Kwun MJ, Croucher NJ, Heller M, Hathaway LJ: **Peptide Occurring in Enterobacteriaceae Triggers *Streptococcus pneumoniae* Cell Death.** *Front Cell Infect Microbiol* 2019, **9:**320.
5. Erni ST, Fernandes G, Buri M, Perny M, Rutten RJ, van Noort JM, Senn P, Grandgirard D, Roccio M, Leib SL: **Anti-inflammatory and Oto-Protective Effect of the Small Heat Shock Protein Alpha B-Crystallin (HspB5) in Experimental Pneumococcal Meningitis.** *Front Neurol* 2019, **10:**570.
6. Muri L, Perny M, Zemp J, Grandgirard D, Leib SL: **Combining Ceftriaxone with Doxycycline and Daptomycin Reduces Mortality, Neuroinflammation, Brain Damage, and Hearing Loss in Infant Rat Pneumococcal Meningitis.** *Antimicrob Agents Chemother* 2019, **63**.
7. Muri L, Le ND, Zemp J, Grandgirard D, Leib SL: **Metformin mediates neuroprotection and attenuates hearing loss in experimental pneumococcal meningitis.** *J Neuroinflammation* 2019, **16:**156.
8. 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.

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