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Institute for Infectious Diseases

Annual Report of the National Center for invasive Pneumococci (NZIP), 2017

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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 (NZIP) 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 NZIP 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 NZIP 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 NZIP in diagnostics and quality assurance.

Routine and special tasks of the NZIP	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**: Differentiation of *S. pneumoniae* from other closely related viridans group streptococci can be challenging. There is no “gold standard” laboratory diagnostic test for the identification of pneumococci and for some atypical isolates a combination of tests has to be applied. The species identification of all strains submitted to the NZIP 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, DNA Probe analysis (AccuProbe *Streptococcus pneumoniae* culture identification test, Gen-Probe, Inc.)).
- **Results**: In 2017 the NZIP received 1’049 isolates recovered from invasive pneumococcal disease (IPD). Thereof 21 isolates were not *S. pneumoniae*

or could not be cultured after transport. Of the *S. pneumoniae* isolates 16 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 1'012 isolates of 1'008 patients were included (two patients had two episodes with the same serotype and two had two different serotypes). The isolates were recovered from blood (n=944), cerebrospinal fluid (n=25), pleural fluid (n=6), synovial fluid (n=9). The remaining 28 isolates derived from other body sites or the origin was not declared by the referring laboratory.

2.1.2 Strain collection

The NZIP stores all the received invasive pneumococcal isolates at -80°C. Collection and storage started in 2002 and currently includes more than 10'000 isolates.

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). A DNA probe analysis and bile solubility test is performed to differentiate these isolates from optochin susceptible non-pneumococcal viridans streptococci. The NZIP 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 2017, 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 2017, the NZIP has received 1'012 strains of *Streptococcus pneumoniae* isolated from normally sterile body sites. The number of isolates increased compared to the previous year, which goes in line with an increase in the number of reported cases at the FOPH (link). In total 48 different serotypes/serogroups were identified in 2017, which is similar to the numbers in previous years (Table 2).

Table 2: Overview of referred IPD isolates 2013-2017

	2013	**2014**	**2015**	**2016**	**2017**
Referred isolates	978	843	898	879	1'012
Number of different serotypes/serogroups	50	46	41	44	48
PCV13 serotype coverage	62.7%	45%	40.9%	37%	33.2%

As in 2016 the five most frequent serotypes were serotype 3 (n=158), serotype 8 (n=150), serotype 22F (n=91), serotype 9N (n=64) and serotype 19A (n=63), which together constituted more than 50% of the invasive isolates (Figures 1 and 2). The proportion of non-PCV13 serotype IPD isolates further increased from 63% in 2016 to 66.8% in 2017 (59.1% in 2015) (Figure 2). The most frequent non-PCV13 serotype responsible for this shift is serotype 8, for which numbers were rapidly increasing from 89 isolates in 2015 (9.9%) to 150 isolates in 2017 (14.8%) (Figure 1B). Likewise, there was an increase of the non-PCV13 serotypes 22F (from 69 isolates in 2015 (7.7%) to 91 isolates in 2017 (9.0%) and 9N (from 33 isolates in 2015 (3.7%) to 64 isolates in 2017 (6.3%).
For the PCV13 serotype isolates on the other hand, no obvious development could be observed over the last three years. The total number of PCV13 isolates was slightly higher than in 2016 (336 in 2017 versus 325 in 2016) but lower than in 2015 (n=367). Serotype 3, although decreasing in 2017 (158 isolates (15.6%) versus 165 isolates (18.8%) in 2016), was still the most frequent serotype. The increasing number of non-PCV13 serotype

isolates was, however, overall causing a further relative reduction of the coverage of PCV13 serotypes compared to the previous years (Table 2).

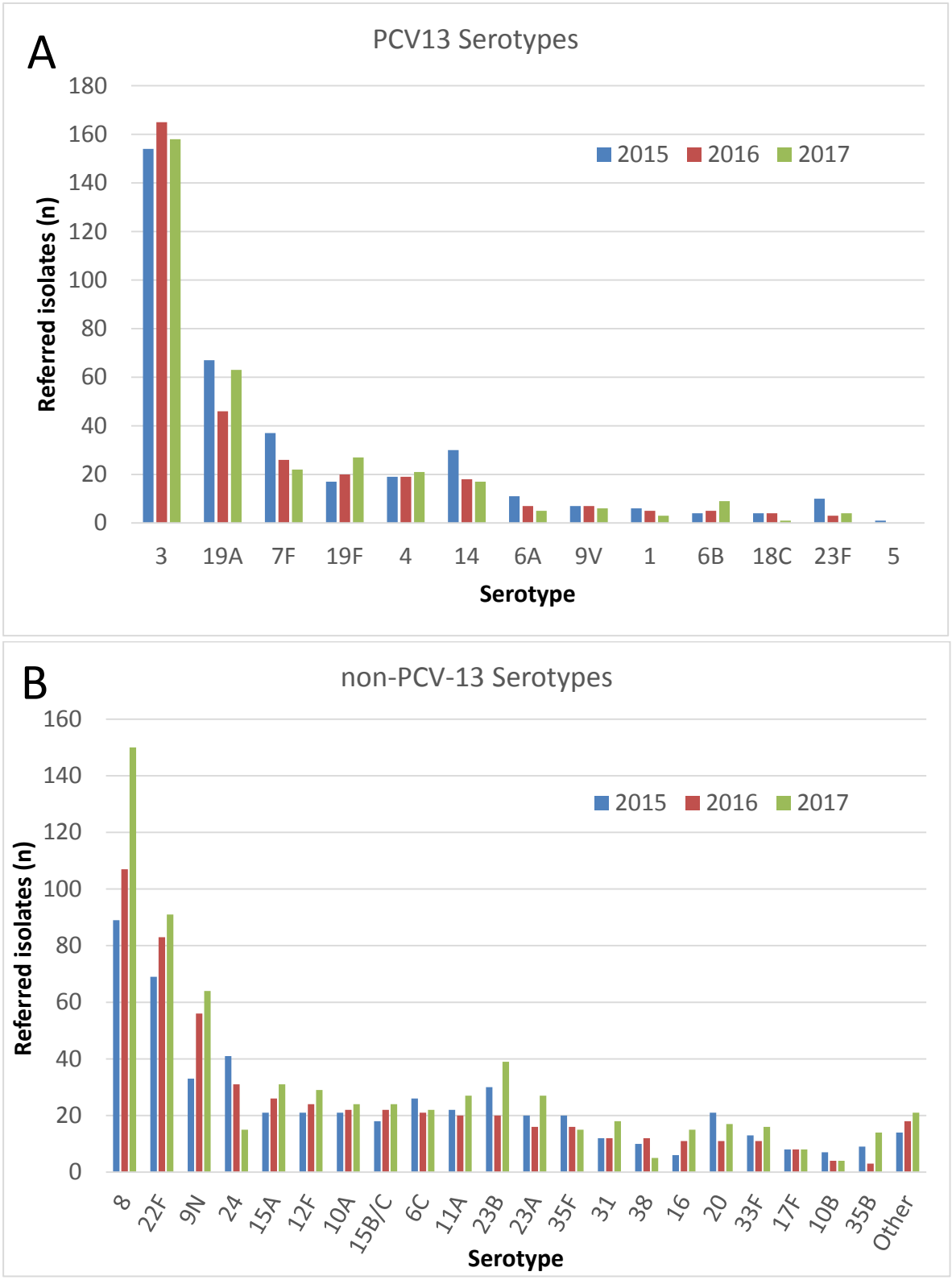


Figure 1: Serotype distribution of invasive *S. pneumoniae,* absolute frequencies in 2017 (1'012 isolates in total) compared to 2015 (898 isolates) and 2016 (879 isolates). (A) PCV13 serotypes; (B) non-PCV13 serotypes.

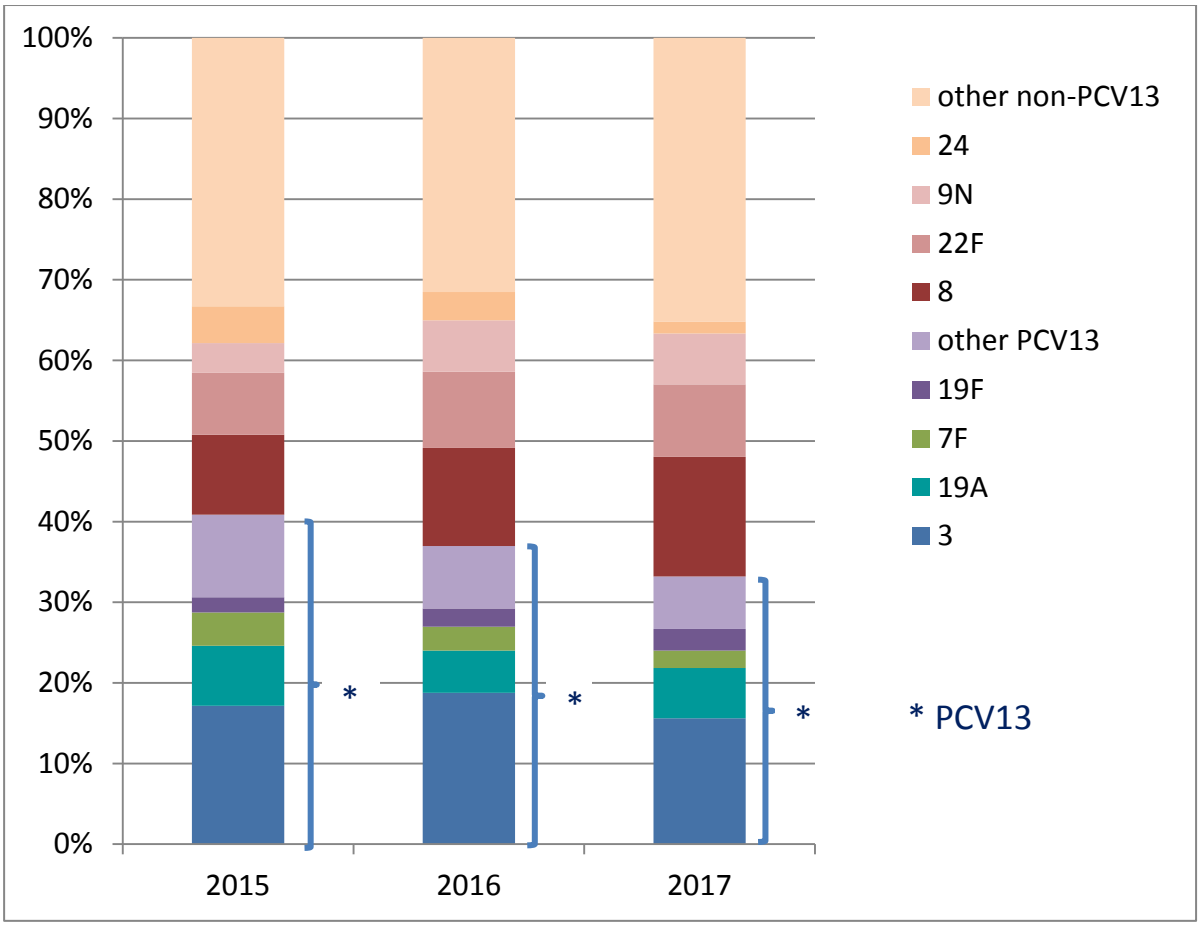


Figure 2: Relative frequencies of most prevalent IPD serotypes in Switzerland in 2015 – 2017

- **Conclusion**: The proportion of PCV13 serotypes in IPD is continuously decreasing since the introduction of the complementary PCV13 vaccination for children under the age of 5 years in 2011. Currently, this decrease appears to be, however, caused mainly by an increase in the absolute numbers of non-PCV13 serotype isolates – most notably serotype 8.

2.1.4 Antibiotic resistance data of invasive pneumococcal isolates

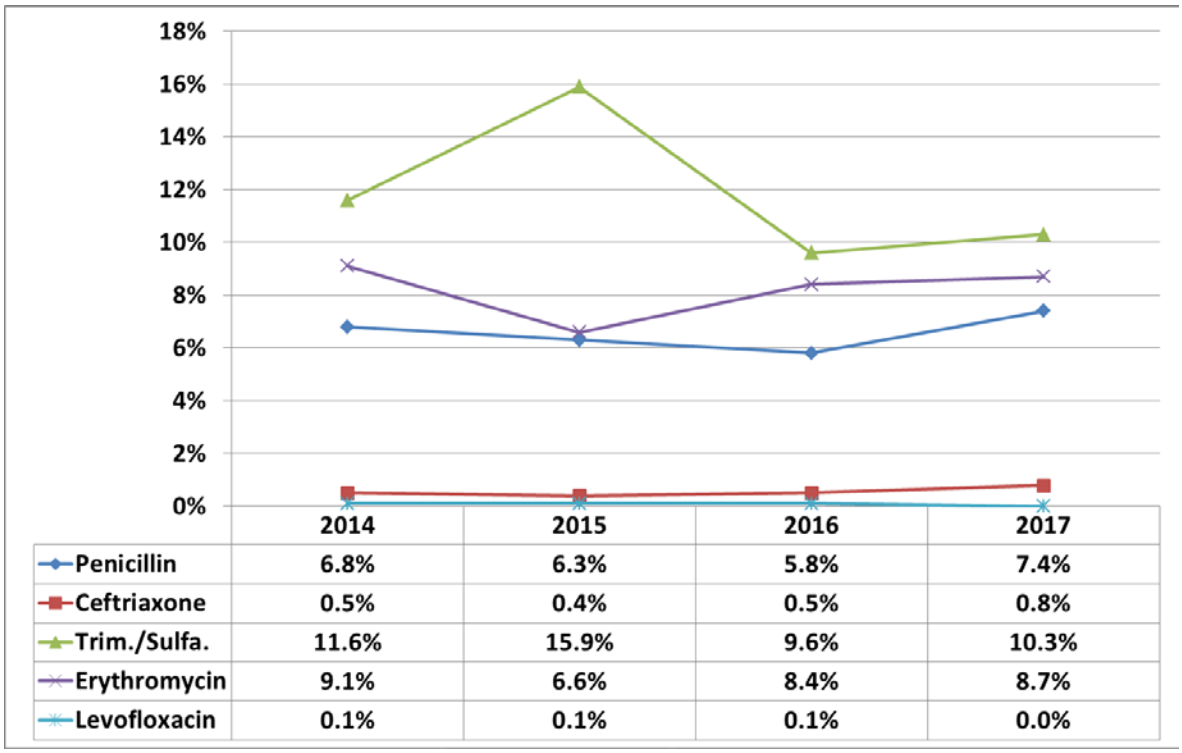
- **Method**: Antibiotic testing includes disk diffusion tests and, for isolates non-susceptible by oxacillin disk screen, minimal inhibitory concentration (MIC) determination by E-tests (bioMérieux, France and Liofilchem, Italy). Values of the E-test on Mueller-Hinton 5% sheep blood agar are interpreted according to Clinical and Laboratory Standards Institute (CLSI) 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 we report the MIC for penicillin and ceftriaxone (interpretive criteria shown in Table 3).

From January 2018, the NZIP is testing the antimicrobial susceptibility according to the guidelines of The European Committee on Antimicrobial Susceptibility Testing - EUCAST.

Table 3: Interpretive standards for *S. pneumoniae* according to CLSI

Antimicrobial Agent	Disk Content	Zone Diameter Interpretive Criteria (mm)			MIC Interpretive Criteria (μg/mL)			
		S	I	R	S	I	R	
Penicillin	1μg oxacillin	≥20	-	-	-	-	-	Corresponds to MIC ≤0.06μg/mL
Penicillin parenteral (meningitis)		-	-	-	≤0.06	-	≥0.12	
Penicillin parenteral (nonmeningitis)		-	-	-	≤2	4	≥8	
Ceftriaxone (meningitis)		-	-	-	≤0.5	1	≥2	
Ceftriaxone (nonmeningitis)		-	-	-	≤1	2	≥4	
Erythromycin	15μg	≥21	16-20	≤15				
Levofloxacin	5μg	≥17	14-16	≤13				
Trimethoprim-sulfamthoxazole	1.25/ 23.75 μg	≥19	16-18	≤15				

- **Results:** In 2017, 75 isolates had a penicillin MIC of >0.06 μg/mL (7.4%). Compared to the previous years the proportion of penicillin non-susceptible isolates was thus slightly higher than in previous years (6.8% in 2014, 6.3% in 2015, 5.8% in 2016, Figure 3). We did, however, not receive any isolates with an MIC >2 μg/ml (i.e. non-susceptible by nonmeningitis criteria). Eight isolates (0.8%) had a ceftriaxone MIC of 0.5μg/mL or higher, i.e. were non-susceptible using the meningitis criteria. For trimethoprim-sulfamethoxazole, 104 isolates revealed non-susceptibility (10.3 % versus 9.6% in 2016) while for erythromycin this was the case for 88 isolates (8.7% versus 8.4% in 2016, 6.6 % in 2015; 9.1% in 2014). All isolates were susceptible to levofloxacin.
  The proportion of isolates that were fully susceptible to all tested antibiotic compounds only slightly decreased from 83.9% in 2016 to 82.2% in 2017. Of the non-susceptible isolates 108 (10.7%) were non-susceptible to one compound, 57 (5.6%) to two and 15 (1.5%) to three compounds.



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

Figure 3: Proportion of non-susceptible IPD isolates (%I or R). For penicillin and ceftriaxone, the meningitis interpretive criteria were applied.

- **Conclusion:** Compared to 2016 the antibiotic resistance prevalence of IPD isolates in Switzerland slightly increased for all antibiotics tested except for levofloxacin, but still remains at a relatively low level.

2.1.5 National and International quality assurance

No international quality assurance was carried out in 2017. The NZIP is going to participate in the next External Quality assurance (EQA) program organized by IBD-labnet / UK NEQAS, which is scheduled for November 2018.

2.1.6 Development of new diagnostic tools

- **Pneumococcal 23B Molecular Subtype Identified Using Whole Genome Sequencing (Publication in 'Genome Biology and Evolution').** We have determined capsule composition in different strains with serotype 23B. On the genomic level, we discovered a new subtype of serotype 23B. However, the newly found subtype did not show a different capsule composition as compared to the other serotype 23B strains. We used a newly set up NMR analysis for this study which is able to differentiate between potentially and 'real' new serotypes. This study has been published in 2017 and is mentioned in the References section below [1].

2.1.7 Epidemiological Research

- **Pneumococcal carriage and serotype variation before and after introduction of pneumococcal conjugate vaccines in patients with acute otitis media in Switzerland (Publication in 'Vaccine').**
  We revealed that pneumococcal carriage declined significantly in Switzerland since 2010, after the introduction of PCV13 in patients with acute otitis media. In addition, PCV13 serotypes generally decreased with the exception of serotype 3. We also recommendend that a surveillance program for non-invasive pneumococci should be in place. This study has been published in 2017 and is mentioned in the References section below [2].
- **Influence of the pneumococcal conjugate vaccines on the temporal variation of pneumococcal carriage and the nasal microbiota in healthy infants: a longitudinal analysis of a case-control study (Publication in 'Microbiome').**
  We performed a case-control study using samples from a longitudinal infant cohort from Switzerland. We compared pneumococcal carriage and the nasal microbiota (i.e. the entity of all microbes) within the first year of life of healthy infants vaccinated with either PCV7 (n = 20, born in 2010) or PCV13 (n = 21, born between 2011 and 2013). Nasal swabs were collected every second week (n = 763 in total). We found a higher number of samples positive for pneumococcal carriage in PCV7- compared to PCV13-vaccinated infants. Also, the nasal bacterial microbiota of infants has changed in recent years as compared to the beginning of this study. Therefore, pneumococcal vaccines influence both, pneumococcal carriage but also carriage and composition of the other bacteria within the nasopharynx. This study has been published in 2017 and is mentioned in the References section below [3].

2.1.8 Additional pneumococcal research

Various additional publications related to pneumococcal meningitis and pneumonia.
We published an article which revealed that the DNA repair protein APE1 is involved in host response during pneumococcal meningitis and its expression can be modulated by vitamin B6 [4]. Furthermore, we discovered an Uncommon Site of *Streptococcus pneumoniae* Colonization Leading to Recurrent Pneumococcal Disease [5]. Finally, we present a novel *ex vivo* experimental setup to examine in detail the pattern of hair cell loss upon exposure to different *S. pneumoniae* strains, therefore recapitulating pathogen derived aspects of pneumococcal meningitis induced hearing loss [6]. 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. If a specific serotype is covered by the used Multiplex PCR, a result can usually be communicated to the clinicians.

3.2 Networking

Public Health England: A research collaboration with the head of the UK Reference Center of Public Healthy England is taking place. We collaborate on how to do serotyping using whole genome sequencing. This has great potential for the future as an additional, diagnostic tool. In addition, the 23B project (see above) took place in close collaboration with the Public Healthy England.

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 2017 in Stockholm, Sweden (http://europneumo.com/home2): We actively took part at the European meeting for Pneumococci and presented our data (oral and 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.

4. Transfer of results

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

The data collected in 2017 were sent to the FOPH on February 20, 2018.

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 2017.

5. Reporting

This report includes data of the NZIP from 2017. 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. *Kapatai G, Sheppard CL, Troxler LJ, Litt DJ, Furrer J, Hilty M, Fry NK:* ***Pneumococcal 23B Molecular Subtype Identified Using Whole Genome Sequencing.*** *Genome Biol Evol 2017,* ***9:****2122-2135.*
2. *Allemann A, Frey PM, Brugger SD, Hilty M:* ***Pneumococcal carriage and serotype variation before and after introduction of pneumococcal conjugate vaccines in patients with acute otitis media in Switzerland.*** *Vaccine 2017,* ***35:****1946-1953.*
3. *Mika M, Maurer J, Korten I, Allemann A, Aebi S, Brugger SD, Qi W, Frey U, Latzin P, Hilty M:* ***Influence of the pneumococcal conjugate vaccines on the temporal variation of pneumococcal carriage and the nasal microbiota in healthy infants: a longitudinal analysis of a case-control study.*** *Microbiome 2017,* ***5:****85.*
4. *Coutinho LG, de Oliveira AHS, Witwer M, Leib SL, Agnez-Lima LF:* ***DNA repair protein APE1 is involved in host response during pneumococcal meningitis and its expression can be modulated by vitamin B6.*** *J Neuroinflammation 2017,* ***14:****243.*
5. *Sendi P, Moser Schaub EM, Nirgianakis K, Hathaway LJ, Bittel P, Goldblatt D, Streit S:* ***An Uncommon Site of Streptococcus pneumoniae Colonization Leading to Recurrent Pneumococcal Disease.*** *Open Forum Infect Dis 2017,* ***4:****ofw257.*
6. *Perny M, Solyga M, Grandgirard D, Roccio M, Leib SL, Senn P:* ***Streptococcus pneumoniae-induced ototoxicity in organ of Corti explant cultures.*** *Hear Res 2017,* ***350:****100-109.*

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