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Institute for Infectious Diseases

Annual Report of the National Center for invasive Pneumococci (NZPn), 2016

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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. Markus Hilty (Research part) under the supervision of Prof. 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**: 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 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, 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 2016 the NZPn received 931 isolates recovered from invasive pneumococcal disease (IPD). Thereof 40 isolates could not be confirmed as *S. pneumoniae*, the majority of these being viridans group Streptococci closely related to *S. pneumonie*, such as *S. mitis* or *S. oralis*. Of the *S. pneumoniae* isolates 12 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 879 isolates of 877 patients were included (One patient had two episodes with two different serotypes and one had two episodes with the same serotype). The isolates were recovered from blood (n=808), cerebrospinal fluid (n=25), pleural fluid (n=8), synovial fluid (n=4), ascites (n=3). The remaining 31 isolates derived from other body sites or the origin was not declared by the referring laboratory.

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.

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 [1]. 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 2016, 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	0				

- **Results**: In 2016, the NZPn has received 879 strains of *Streptococcus pneumoniae* isolated from normally sterile body sites. The number of isolates decreased compared to the previous year, which goes in line with the slightly lower incidence reported by the FOPH (link). In total 44 different serotypes/serogroups were identified in 2016, which is similar to the numbers in previous years (46 different serotypes/serogroups in 2014, 41 serotypes/serogroups in 2015, Table 2).

Table 2: Overview of referred IPD isolates 2013-2016

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

As in 2015 the three most frequent serotypes were serotype 3 (n=165), serotype 8 (n=107) and serotype 22F (n=83) (Figure 1A and B). There was a noticeable increase in serotype 9N, which is now the fourth most frequent serotype (n=56 (6.4%) versus n=33 (3.7%) in 2015). In general, the proportion of non-PCV13 serotype IPD isolates further increased from 59.1% in 2015 to 63% in 2016 (55% in 2014) (Figure 2). The most frequent non-PCV13 serotypes accounting for this increase were besides serotype 9N mainly serotype 8 (increasing from 9.9% in 2015 to 12.2% in 2016) and serotype 22F isolates (7.7% in 2015, 9.4% in 2016). On the other hand, there was a further decrease of the PCV13 serotype isolates 19A (n=46 (5.2%) versus n=67 (7.5%) in 2015) and 7F (n=26 (3%) versus n=37 (4.1%) in 2015). In total, there was thus a further reduction

of the coverage of PCV13 serotypes compared to the previous years (Table 2). Whereas in 2015 we observed an increased number of serogroup 24 isolates the proportion of this serogroup decreased again in 2016 (n=31 (3.5%), n=41 (4.6%) in 2015, n=28 (3.3%) in 2014)

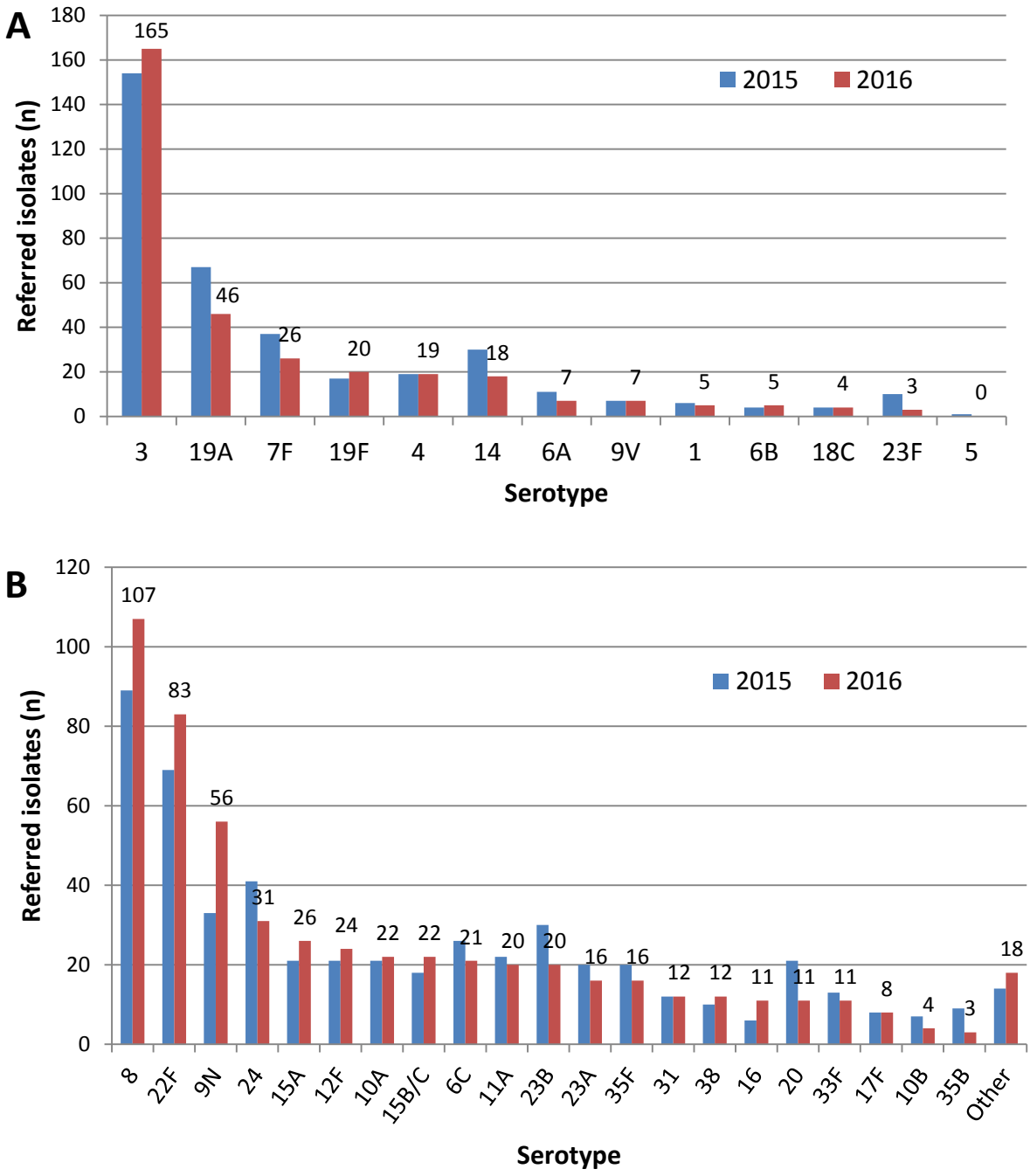

Figure 1: Serotype distribution of invasive *S. pneumoniae,* absolute frequencies in 2016 (879 isolates in total) compared to 2015 (898 isolates). (A) PCV13 serotypes; (B) non-PCV13 serotypes.

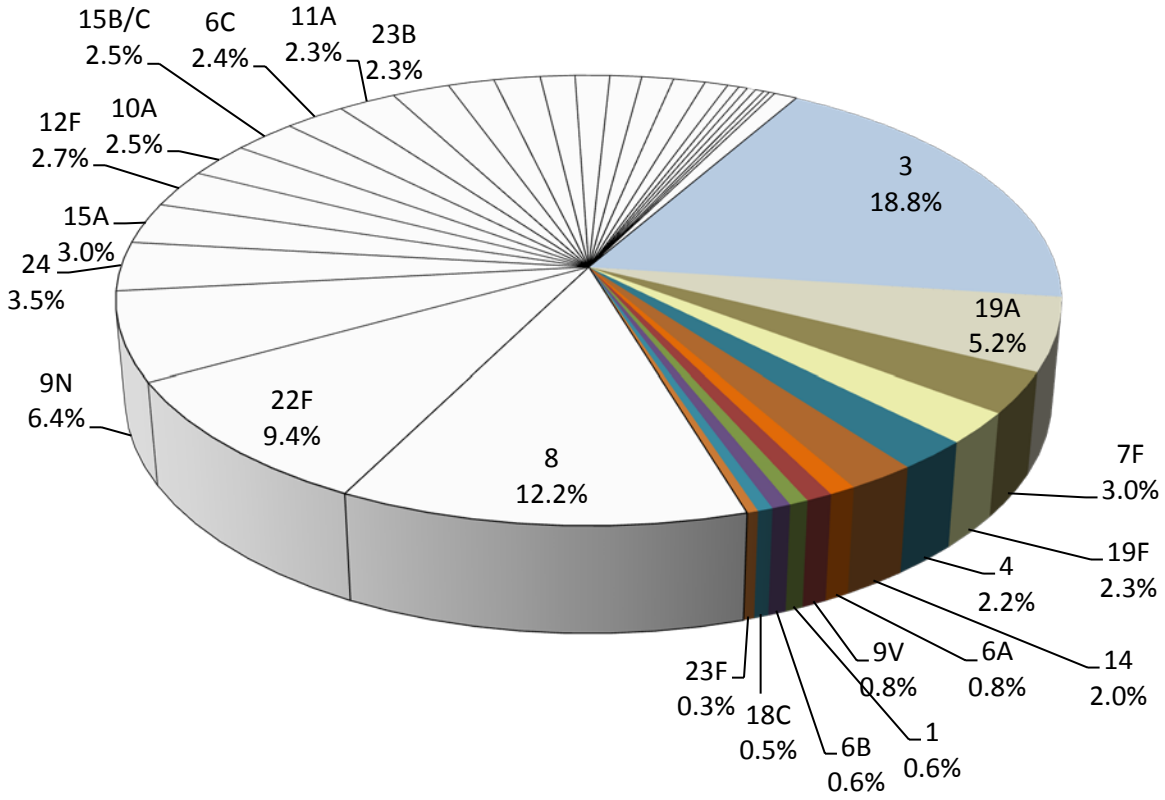

Figure 2: Relative serotype frequencies of invasive *S. pneumoniae* in 2016 (879 strains in total).PCV13 serotypes are indicated in color

- **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. One exception is serotype 3, which is still the most frequent serotype in IPD and whose frequency was again increasing in 2016.

2.1.4 Antibiotic resistance data of invasive pneumococcal isolates

- **Method**: Antibiotic testing includes disk diffusion tests and E-Tests, if non-susceptible by oxacillin disk screen.Values of the E-test (AB Biodisk, Sweden, distributed in Switzerland by Biomérieux) on Mueller-Hinton 5% sheep blood agar are interpreted according to Clinical and Laboratory Standards Institute (CLSI) recommendations. 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).

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 total, 51 isolates had a penicillin MIC of >0.06 µg/mL (5.8%). There was thus a further decrease in penicillin non-susceptibility compared to the previous years (6.8% in 2014, 6.3% in 2015). As in 2015 we did not reveal any isolates with an MIC >2 µg/ml (i.e. non-susceptible by nonmeningitis criteria). For cotrimoxazole, 84 isolates revealed non-susceptibility (9.6 % versus 15.9 % in 2015) while for erythromycin this was the case for 74 isolates (8.4% in 2016 versus 6.6 % in 2015; 9.1% in 2014). One isolate was resistant to levofloxacin. Resistance rates thus decreased for penicillin and cotrimoxazole but, in contrast increased for erythromycin as compared to 2015.

- **Discussion:** In general, the antibiotic resistance prevalence of IPD isolates in Switzerland was lower than in 2015. Whereas in 2015 20.3% of the isolates were non-susceptible to at least one antibiotic compound, the proportion of non-susceptible isolates decreased to 16.1% in 2016. In 2016 7.6% of PCV13 isolates had an MIC >0.06 for penicillin; whereas this was the case for only 4.7% of non-PCV13 isolates. The reduction of PCV13 serotype isolates could therefore be a possible cause for the lower resistance towards penicillin (see also Ref. [3]). Only for erythromycin we observed an increase in the resistance rate, which however was still lower than in 2014 (9.1% of isolates non-susceptible). For 2015 we reported a high number of serogroup 24 isolates, for which an association with cotrimoxazole resistance has been observed. In 2016

the number of serogroup 24 isolates and thereby the overall cotrimoxazole resistance rate decreased again.

2.1.5 National and International quality assurance

No international quality assurance was carried out in 2016. However, the NZPn is going to participate in the next External Quality assurance (EQA) program organized by IBD-labnet / UK NEQAS.

2.1.6 Development of new diagnostic tools

- **Polysaccharide Capsule Composition of Pneumococcal Serotype 19A Subtypes Is Unaltered among Subtypes and Independent of the Nutritional Environment (Publication in Infection and Immunity).** We have determined capsule composition in different nutritional conditions with high-performance liquid chromatography (HPLC), gas chromatography - mass spectrometry (GC-MS) and nuclear magnetic resonance spectroscopy (NMR). Therefore, we have optimized these techniques as new diagnostic tools. However, an NMR analysis was most superior in being able to differentiate between potentially and 'real' new serotypes. This study has been published in 2016 and is mentioned in the References section below [2].

2.1.7 Epidemiological Research

- **Serotype/serogroup-specific antibiotic non-susceptibility of invasive and non-invasive *Streptococcus pneumoniae*, Switzerland, 2004 to 2014 (Publication in Eurosurveillance).** We have published a major epidemiological study analyzing the antimicrobial resistance in non-invasive and invasive pneumococcal isolates. In more detail, we revealed that antibiotic resistance rates within IPD were on an all-time low for < 5 years old (erythromycin), 5-64 years old (penicillin, erythromycin, cotrimoxazole) and >64 years of age (cotrimoxazole) in the most recent years. Furthermore, decreasing resistance rates were due to the decrease of more resistant serotypes due to the introduction of PCV7 and PCV13. However, we also concluded that certain non-PCV13 serotypes prone to carry resistance have to be still carefully monitored in the future. This study has been published in the EUROSURVEILLANCE, the official journal of the European Center for Disease Control and is mentioned in the References section below [3].

- **Pneumococcal carriage and serotypes distribution in patients with acute otitis media in Switzerland, 2004-2014. (Poster presentation at ECCMID 2016 (DOI).** 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.

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 including all PCV13 serotypes. If a specific serotype is covered by the used Multiplex PCR, a result can usually be communicated to the clinicians. The muliplex PCR is very specific for the 28 included serotypes/serogroups and very sensitive as it is a DNA based method [4]. Therefore, its advantage is the applicability if no culture is available but, as a disadvantage, it can only detect 28/92 serotpyes.

Antimicrobial susceptibility testing: In 2016 there was a recall of several batches of commercial Mueller Hinton +5% horse blood + 20mg/l beta-NAD (MH-F) media (https://www.swissmedic.ch/recalllists_dl/13865/Vk_20160615_14-En1.pdf). The NZPn exchanged results and *S. pneumoniae* strains with referring laboratories in order to address the problem of inconsistent results for cotrimoxazole susceptibility testing on various MH-F media

3.2 Networking

Public Health England: At the bi-yearly ISPPD (DOI), an exchange with the head of the UK Reference Center of Public Healthy England took place. We will collaborate on how to do serotyping using whole genome sequencing. This has great potential for the future as an additional, diagnostic tool.

4. Transfer of results

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

The data collected in 2016 were sent to the FOPH on February 16, 2017.

4.2. Transfer of results to the referring laboratories

Serotyping and antimicrobial susceptibility testing results are normally sent to the referring laboratories within one week at the most. No irregularities have occurred during 2016.

5. Reporting

This report includes data of the NZPn from 2016. 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. References

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2. *Brugger SD, Troxler LJ, Rufenacht S, Frey PM, Morand B, Geyer R, Muhlemann K, Hock S, Thormann W, Furrer J, et al:* ***Polysaccharide Capsule Composition of Pneumococcal Serotype 19A Subtypes Is Unaltered among Subtypes and Independent of the Nutritional Environment.*** *Infect Immun 2016,* ***84:****3152-3160.*
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4. *Pai R, Gertz RE, Beall B:* ***Sequential multiplex PCR approach for determining capsular serotypes of Streptococcus pneumoniae isolates.*** *J Clin Microbiol. 2006 Jan;****44****(1):124-31*

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