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Annual Report of the National Center for invasive Pneumococci (NZPn), 2015

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1. Organization

Since 1 March 2002, the institute for infectious diseases hosts the National Center for invasive Pneumococci (NZPn) which is funded 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*. From 2002-2012, the center was led by Prof. Kathrin Mühlemann. Since then, PD. Dr. Markus Hilty was in charge. As for July 2016 onwards, the NZPn will be co-led by Dr. phil. Carlo Casanova (Diagnostic and administrative part) and PD. Dr. Markus Hilty (Research part). With a closer integration of the diagnostic with the research part, a more optimized running of the center is envisaged.

2. Diagnostic and quality assurance

Among the tasks of NZPn are confirmatory diagnostics of 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” 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 2015, NZPn received 938 invasive samples. However, one isolate no bacterial growth was achieved and 26 samples were viridans streptococci. The viridans streptococci are a large group of commensal streptococcal bacteria species that are either α-hemolytic, producing a green coloration on blood agars or nonhemolytic. Therefore 26/938 samples (2.77 %) were 'wrong positive' for pneumococci. Finally, 13 pneumococcal isolates were excluded as they were 'doubles'. Therefore, the final analysis included 898 isolates (see below).

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 10000 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 experiences with PCV7 suggest 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 immediately 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 2015, no additional adaptions have been made. This means that we currently test for the following serorgroups/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	26	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						

* Non-typeable means that the isolate could have a capsule which is non-typeable with the sera currently used at the NZPn. It could also mean that the isolate has no capsule at all. However, as for the latter, we normally call the isolate as beeing non-encapsulated rather than non-typeable.

- **Results**: During the year 2015, the NZPn has received 898 strains of *Streptococcus pneumoniae* isolated from normally sterile specimens like blood (n=837) and other sites (n=61). This number is higher than in 2014 (n=843) but less than in 2013 (n=978). 898 strains accounted for 41 different serotypes/serogroups. The number of different serotypes within Switzerland is lower as compared to the previous years (46 different serotypes/serogroups in 2014; and 50 in 2013). This could mean that there is a consolidation of existing, probably well 'adapted' serotypes taking place. This is in contrast to the first years after PCV13 introduction, when it seemed that mainly diversification of serotypes took place.
  However, the serotype 3 (n=154) is still the most frequent, serogroup 8 (n=89) and serotype 22F (n=69) are now second and third, respectively (in 2014 first, second and third were serotypes 3 (n=131), 22F (n=91) and 8 (n=59), respectively) (Figure 1A). Overall, 367 strains were covered by PCV13 (40.9%) (Figure 1B) while 379 strains were covered by PCV13 (45.0%) in 2014. Therefore, a reduction of the coverage of PCV13 serotypes compared to the previous year has been noted which, however, was not as drastic as compared to 2013 (62.7% in 2013). In contrast, non PCV13 serotypes have increased with serogroup 8 and serotype 22F being the most frequent non PCV13 serotypes in 2015 (Figure 1A and 1B). Of special note is the increased number of serogroup 24 isolates (n=41 (4.6%) versus n=28 (3.3%) in 2014). Serogroup 24 isolates are important due to cotrimoxazol non-susceptibility (see below).
  Discussion of the Results: In 2015, we could detect an increase of submitted *S. pneumoniae* isolates compared to 2014. This finding was unexpected but is probably a temporary increase as in general it looks like a decrease has taken place since 2011 when the complementary vaccination with PCV13 was introduced for children under the age of 5 years. Of course, incidence numbers give a clearer picture than exclusively counting the received isolates. Checking the FOPH website (DOI), however, revealed that there was indeed an increase of the incidence of IPD in 2015 (10.6/1000000) as compared to 2014 (9.6/100000). However, the number of isolates with non-PCV13 serotypes are arising which is not true for the number of different serotypes.

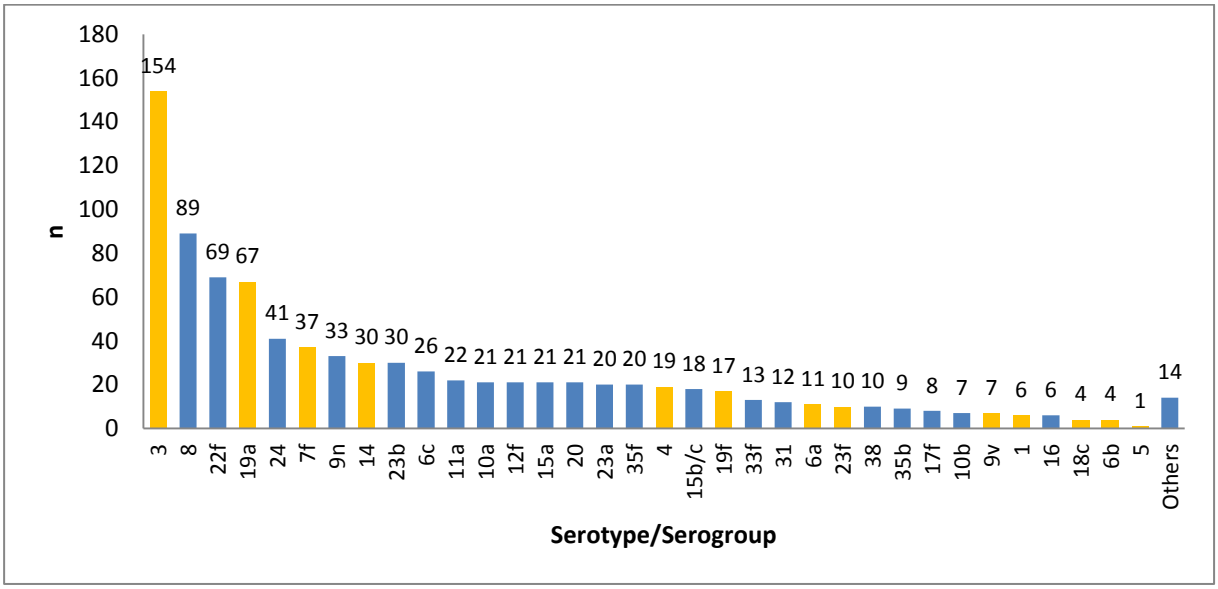

Figure 1A: Absolute frequency of invasive *S. pneumoniae,* 2015 (898 strains in total)*

*PCV13 serotypes are indicated in orange

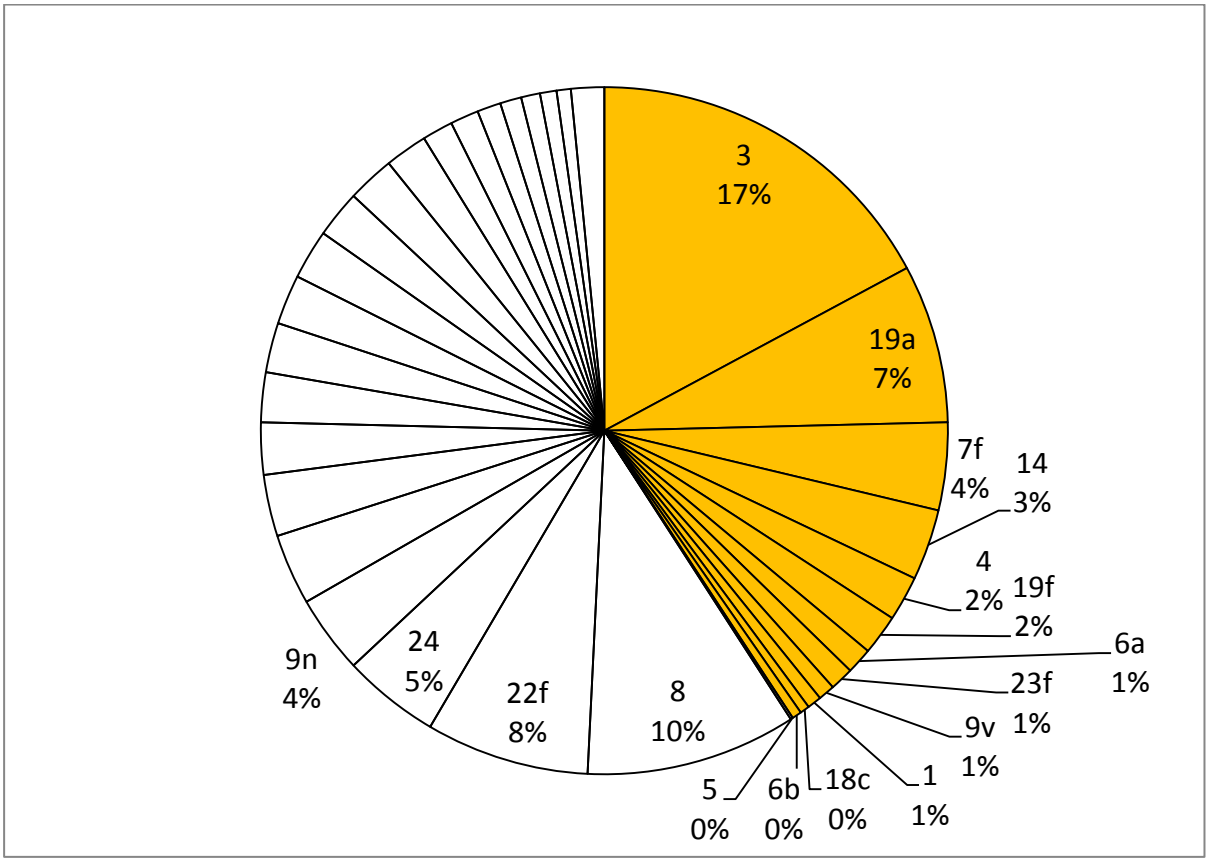

Figure 1B: Relative frequency of invasive *S. pneumoniae,* 2015 (898 strains in total)*

*PCV13 serotypes are indicated in orange

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 minimal inhibition concentration (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 2).

Table 2: 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, 57 isolates weren't susceptible to penicillin as they showed an MIC of >0.06 µg/mL towards penicillin (6.3%). Penicillin resistance is therefore slightly lower as compared to 2014 (6.8%). We did not reveal any isolates with a MIC >2 µg/ml (i.e. non-susceptible according to criteria applied in case of invasive pneumococcal disease without meningitis). As for cotrimoxazol, 143 isolates (15.9 %; 11.6 % in 2014) revealed non-susceptibility while for erythromycin this was true for 59 isolates (6.6 %; 9.1% in 2014). Two isolates revealed non-susceptibility towards levofloxacin. Therefore, resistance rates dropped for penicillin and erythromycin but, in contrast increased for cotrimoxazol as compared to 2014. Interestingly a particular high number of isolates of the non-PCV13 serogroup 24 (35 of 41 (85%)) were non-susceptible to cotrimoxazol.
- **Discussion:** As for antibiotic testing for Penicillin G, the NZPn reports all isolates with a MIC >0.06 µg/ml as non-susceptible. In general, the antibiotic resistance prevalence in 2015 is lower as the ones from 2014. This is,

however, in contrast to cotrimoxazol for which a high number of serogroup 24 isolates are non-susceptible. This is slightly worrying as serogroup 24 is not covered by PCV13 but numbers are still low as it was recently shown (for serotype 24F) in Germany, too [2, 3]

2.1.5 National and International quality assurance

No international quality assurance was done in 2015. However, the NZPn is in contact with the person in charge for such an External Quality assurance (EQA) program. We have been told that Participation of Non-Member State or Non-EEA counties in EQA schemes organized by IBD-labnet is in principal possible – under the understanding that these countries will cover the costs for their participation. As for the next program, the next EQA distributions are scheduled for 2017 and 2019, respectively. Therefore, the NZPn will take part in 2017.

2.1.6 Development of new diagnostic tools

At the NZPn, we hypothesize that pneumococcal vaccine effectiveness against serotype 19A and 19A serotyping accuracy might be impaired by structural differences in serotype 19A capsules. We therefore 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). However, so far, chemical analyses showed no difference in the capsule composition. The capsule consisted of a trisaccharide repeat unit composed of rhamnose, N-acetyl-mannosamine and glucose, which is the normal composition for serotype 19A. However, NMR analyses will help to also find ‘new’ serotypes in the future and may therefore serve as a new diagnostic tool. A study to this topic will be submitted in 2016.

2.1.7 Research

In 2015, we had the following 2 poster presentations:

- **Dynamics of serotype/serogroup specific antibiotic resistance of Streptococcus pneumoniae in Switzerland (2004-2013) (Poster presentation at ECCMID 2015 (www.eccmid.org)). DOI**
  We revealed that, 2013 antibiotic resistance rates within IPD are on an all-time low for < 5 years old (erythromycin), 5-64 years old (penicillin, erythromycin, cotrimoxazole) and >64 years of age (cotrimoxazole). Furthermore, decreasing resistance rates were attributable to the reduction of more resistant serotypes due to the introduction of PCV7 and PCV13. Certain non-PCV13 serotypes prone to carry resistance have to be carefully monitored in the future.

- **Burden of invasive pneumococcal disease (IPD) and serotype epidemiology, Switzerland, 2005-2014 (Poster presentation at Swiss Society for Infectious Disease meeting (SGINF 2015)). DOI**
  A reduction of IPD incidence by 61.2% in young children and by 22.0% in adults was shown for Switzerland. In older children 5-14 years of age which were not yet routinely vaccinated with PCV13 there was no significant reduction of overall-IPD incidence. The reductions in IPD are largely mirrored by PCV13-serotype reduction. However, some non-vaccine Serotypes/groups (15, 22F, 24, 23A/B) were shown to be emerging and warrant close surveillance.

3. Advisory service and networking

3.1 Advisory service

Molecular test: In 2015, the NZPn received three samples for which serotyping was done using a molecular test (Multiplex PCR). DNA samples were sent from the Kantonsspital Lucerne. Our current Muliplex PCR approach covers 28 different serotypes/serogroups. If the serotype is covered by the used Multiplex PCR, a result can normally be communicated to the clinicians.

3.2 Networking

German Reference Center: At the yearly ECCMID, a regular exchange with the head of the German Reference Center has taken place (M. van der Linden). During the meeting, latest advancement on new diagnostic tools and the evaluation of the serotype epidemiology, among other issues, were discussed. Serotype and antibiotic epidemiology is normally comparable with the German setting.

Austrian Reference Center: In 2013, the Austrian Centre sent four pneumococcal isolates which were ‘difficult to serotype’. However, after performing serotyping at NZPn, results were concordant. In 2015, no such exchange was done. However, as for 2016, this is again planned.

4. Transfer of results

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

Data were sent to the FOPH according to the specifications outlined on 21.1.2016.

4.2. Transfer of results to the laboratories

Analysis results are normally sent to the laboratories within one week at the most. No irregularities have occurred during 2015

5. Reporting

This report includes data of the NZPn from 2015. 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. Teaching

A poster presentation to infectious disease specialists and physicians was held at the Swiss Society for Infectious Diseases meeting at Interlaken (1.9-4.9.2015).

7. Research projects with partners

Research co-operations were ongoing with the Sanger institute for the sequencing of pneumococcal isolates and the Austrian Reference Center for pneumococci for the detection of 'challenging' serotypes.

8. References

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As for this report:

PD Dr. phil nat Markus Hilty (and Dr. phil. Carlo Casanova)