ECCMID 2018
Poster #P0606

Changes in Serotype Distribution and Antimicrobial Nonsusceptibility in *Streptococcus pneumoniae* Causing Pneumonia in Adults from 4 European Countries Following Paediatric Immunization with 13-Valent Pneumococcal Conjugate Vaccine (PCV13)

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Presented at ECCMID, 2018. 28th European Congress of Clinical Microbiology and Infectious Diseases. Poster Session #: PS34, "*S. pneumoniae*" Poster #0606. Date: April 22, 2018. Madrid, Spain

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Introduction

- Paediatric routine use of 13-valent pneumococcal conjugate vaccine (PCV13) has been associated with substantial declines in the proportion of vaccine-type pneumococcal pneumonia in adults
  - This decline was likely caused by the indirect protection due to the reduced transmission of pneumococci from vaccinated children to unvaccinated adults
- Continued monitoring of the impact of infant vaccination in preventing vaccine-type pneumococcal disease in adults is required to assess the additional impact of PCV13 use in adults
- This study assessed changes in serotype distributions and antimicrobial nonsusceptibility patterns in pneumococcal isolates obtained from adults aged ≥50 years with documented pneumonia in 4 European countries (the United Kingdom, France, Germany, and Ireland) with routine paediatric PCV13 use

Materials and Methods

Clinical isolates

- This study utilized 670 isolates that were recovered during the periods of pre- (2009–2010) and post-PCV13 (2015–2016) immunization programs
  - Isolates were recovered from adult patients (≥50 years old) seen or hospitalized in 21 medical centres located in 4 European countries (the United Kingdom, France, Germany, and Ireland) during 2009–2010 (n=311 isolates) and 2015–2016 (n=359 isolates)
- Isolates were collected primarily from invasive (22.5%) and lower respiratory tract specimens (77.5%) of patients with documented pneumonia and submitted to a central monitoring laboratory (JMI Laboratories, North Liberty, Iowa, USA) as part of the SENTRY Antimicrobial Surveillance Program
- Participating clinical laboratories identified bacteria and the central monitoring laboratory confirmed bacterial identification using standard microbiologic techniques
- Isolates were subjected to a PCR assay for further identification when bacterial identification was questionable after using phenotypic methods or an untypeable serotyping result was obtained by the applied methodology

Antimicrobial susceptibility testing

- Antimicrobial susceptibility testing of isolates was performed by broth microdilution following guidelines from the Clinical and Laboratory Standards Institute (CLSI) M07 document (2018)
- Quality assurance was performed by concurrently testing CLSI-recommended quality-control (QC) reference strains (*S. pneumoniae* ATCC 49619)
  - All QC results were within published acceptable ranges
- Breakpoint criteria were those from EUCAST (2018)

Pneumococcal serotyping

- The *cpsB* gene was sequenced by PCR assays or whole genome sequencing
- Nucleotide sequences were analysed using the Lasergene software package (DNASTAR, Madison, Wisconsin, USA)
- Sequences were compared to others available via NCBI (http://www.ncbi.nlm.nih.gov/blast/)
- Due to sequence homology among certain serotypes, those showing nucleotide sequence similarity greater than 99% were grouped (e.g., 9V/9A, 7F/7A, 11A/11D, 15A/15F, 22F/22A, 15B/15C)
- All isolates determined to be serogroup 6 by sequencing analysis were subjected to multiplex PCR assays for confirmation and discrimination between 6A/6B and 6C/6D
- Isolates determined to be serogroup 6A/6B and 7F/7A were serotyped by Quellung reaction using commercially available antisera according to manufacturer's instructions (Statens Serum Institut, Copenhagen, Denmark)

Multilocus sequence typing

- Multilocus sequence typing (MLST) was performed by extracting the previously defined set of 7 housekeeping gene fragments (~500 bp) for *S. pneumoniae*
  - Each fragment was compared to known allelic variants for each locus (housekeeping gene) on the MLST website (PubMLST, https://pubmlst.org/spneumoniae/)
- An allele sharing 100% genetic identity with a known variant received a number designation and a 7 number sequence (1 for each housekeeping gene) formed an allelic profile, defined as sequence types (STs)
  - Isolates having alleles that did not match an existing sequence in the MLST database were submitted/deposited for allele and ST assignments
- ST profiles sharing 100% genetic identity in at least 5 of 7 MLST loci were grouped into a clonal complex (CC) named after its presumed ancestral genotype

Results

- Among pneumococcal pneumonia cases, PCV7-type dropped from 21.9% to 4.2% and PCV13-type dropped from 54.3% to 21.2% between 2009–2010 and 2015–2016 (p<0.0001 for both PCV) (Table 1 and Figure 1A)
- Distribution of serotype 19 remained similar (9.3% vs. 9.5%) overall, but dropped from 10.0% to 6.4% when excluding Ireland; while prevalence of serotype 3, 7F and 6A decreased, regardless of region analyzed (Table 1)
- The majority of the serotype 19A cases (85.3%) in 2015–2016 were from France (41.2%) and Ireland (44.1%) and they represented 11.2% and 23.8% and they accounted for 11.2% and 23.8% of the pneumococcal pneumonia cases in those countries

Table 1 Serotype Distribution of *S. pneumoniae* Pneumonia in Adults ≥50 Years over Time

	Number (%) of *S. pneumoniae* serogroups/types			
	All countries		Ex-Ireland	
Serogroup/type[a]	2009–2010 (311)	2015–2016 (359)	2009–2010 (259)	2015–2016 (296)
PCV7-type	68 (21.9)	15 (4.2)	46 (17.8)	13 (4.4)
19F	20 (6.4)	10 (2.8)	12 (4.6)	9 (3.0)
9V/9A	11 (3.5)	0 (0.0)	9 (3.5)	0 (0.0)
6B	10 (3.2)	3 (0.8)	6 (2.3)	3 (1.0)
23F	10 (3.2)	1 (0.3)	8 (3.1)	1 (0.3)
4	2 (0.6)	0 (0.0)	2 (0.8)	0 (0.0)
18 (18A/18B/18C/18F)	3 (1.0)	1 (0.3)	2 (0.8)	0 (0.0)
14	12 (3.9)	0 (0.0)	0 (0.0)	0 (0.0)
PCV13-type	169 (54.3)	76 (21.2)	135 (52.1)	57 (19.3)
PCV13 minus PCV7-type[a]	101 (32.5)	61 (17.0)	89 (34.4)	44 (14.9)
19A	29 (9.3)	34 (9.5)	26 (10.0)	19 (6.4)
3	39 (12.5)	24 (6.7)	38 (14.7)	22 (7.4)
7F	12 (3.9)	2 (0.6)	10 (3.9)	2 (0.7)
6A	16 (5.1)	1 (0.3)	10 (3.9)	1 (0.3)
1	4 (1.3)	0 (0.0)	4 (1.5)	0 (0.0)
5	1 (0.3)	0 (0.0)	1 (0.4)	0 (0.0)
PPV23-type unique[b]	58 (18.6)	106 (29.5)	54 (20.8)	84 (28.4)
11A/11D	16 (5.1)	21 (5.8)	13 (5.0)	15 (5.1)
22A/22F	11 (3.5)	11 (3.1)	11 (4.2)	9 (3.0)
15B/15C	7 (2.3)	11 (3.1)	7 (2.7)	10 (3.4)
9N/9L	4 (1.3)	16 (4.5)	4 (1.5)	13 (4.4)
10A	7 (2.3)	11 (3.1)	7 (2.7)	10 (3.4)
33F/33A	4 (1.3)	7 (1.9)	4 (1.5)	6 (2.0)
17F	5 (1.6)	10 (2.8)	4 (1.5)	7 (2.4)
8	3 (1.0)	8 (2.2)	3 (1.2)	7 (2.4)
20	1 (0.3)	4 (1.1)	1 (0.4)	2 (0.7)
12F/12A/44/46	0 (0.0)	7 (1.9)	0 (0.0)	5 (1.7)
Non-vaccine-type[c]	84 (27.0)	172 (47.9)	70 (27.0)	151 (51.0)
35B	21 (6.8)	34 (9.5)	15 (5.8)	24 (8.1)
23A	7 (2.3)	22 (6.1)	6 (2.3)	20 (6.8)
6C/6D	7 (2.3)	10 (2.8)	6 (2.3)	8 (2.7)
15A/15F	11 (3.5)	27 (7.5)	10 (3.9)	24 (8.1)
23B	10 (3.2)	16 (4.5)	8 (3.1)	14 (4.7)
16F	4 (1.3)	8 (2.2)	4 (1.5)	7 (2.4)
31	5 (1.6)	13 (3.6)	3 (1.2)	13 (4.4)
35F/47F	6 (1.9)	13 (3.6)	5 (1.9)	13 (4.4)
7C/7B/40	0 (0.0)	1 (0.3)	0 (0.0)	1 (0.3)
34	1 (0.3)	2 (0.6)	0 (0.0)	2 (0.7)
21	5 (3.6)	3 (0.8)	5 (1.9)	3 (1.0)
38/25F/25A	2 (0.6)	0 (0.0)	1 (0.4)	0 (0.0)
24	3 (1.0)	5 (1.4)	3 (1.2)	5 (1.7)
Other	4 (1.3)	18 (5.0)	4 (1.5)	0 (0.0)
Untypeable	0 (0.0)	5 (1.4)	0 (0.0)	4 (1.4)

[a] PCV7 includes serotypes 4, 6B, 9V, 14, 18C, 19F, and 23F.
PCV13-PCV7 includes serotypes 1, 3, 5, 6A, 7F, and 19A.
PCV13 includes serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F.
[b] Includes PPV serotypes (2, 8, 9N, 10A, 11A, 12F, 15B, 17F, 20, 22F, and 33F) and excludes PCV13 serotypes.
[c] Nonvaccine serotypes are those not included in PCV and/or PPV.

Figure 1A. Changes in Serotype-Grouping Distribution of *S. pneumoniae* Pneumonia in Adults ≥50 Years over Time
Ireland, France, Germany, and U.K. Combined

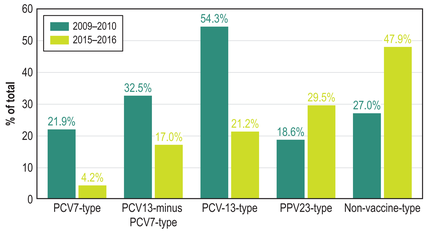
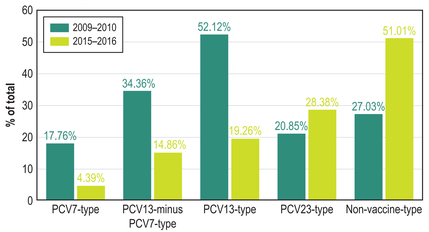


Figure 1B. Changes in Serotype-Grouping Distribution of *S. pneumoniae* Pneumonia in Adults ≥50 Years over Time
France, Germany, and U.K. Combined



PCV13 introduced in Germany in 2009 and in France, Ireland, and the United Kingdom in 2010.
PCV7 includes serotypes 4, 6B, 9V, 14, 18C, 19F, and 23F.
PCV13-PCV7 includes serotypes 1, 3, 5, 6A, 7F, and 19A.
PCV13 includes serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F.
PCV23 includes PPV serotypes (2, 8, 9N, 10A, 11A, 12F, 15B, 17F, 20, 22F, and 33F) and excludes PCV13 serotypes.
Nonvaccine serotypes are those not included in PCV and/or PPV.

PPV23-type and non-vaccine-type disease

- Distribution of PPV23 unique serotypes (those serotypes in PPV23 but not in PCV13) in pneumococcal pneumonia increased from 18.6% to 29.5% between 2009–2010 and 2015–2016 (Table 1 and Figure 1A)
- Non-vaccine types (neither PPV23-type nor serotype 6A-type) increased from 27.0% to 47.9% (p<0.0001) between both study periods (Table 1 and Figure 1A)
- Among non-vaccine-type, serotype 35B accounted for 9.5% of pneumococcal pneumonia in 2015-2016 (Table 1)

Antimicrobial non-susceptibility

- Non-susceptibility rates for penicillin (parenteral; MIC >2 mg/L), ceftriaxone, clindamycin, and erythromycin in pneumococcal pneumonia were lower in 2015–2016 compared to 2009–2010 (Figure 2A)
- Non-susceptibility in PCV7-type pneumococcal pneumonia for penicillin decreased from 14.7% to 0.0% (p<0.01) but increased in PCV13-type from 7.1% to 14.5% between 2009–2010 and 2015–2016. The observed increase in non-susceptibility in PCV13-type disease was caused by serotype 19A (11 of 13 cases) clustered within clonal complex CC320 (10 cases of ST320 and 1 case of ST6398, where ST6398 is a single locus variant of ST320) from 2 medical sites in Dublin and Galway
- When excluding Ireland from the analysis, antimicrobial non-susceptibility rates for penicillin, ceftriaxone, clindamycin, and erythromycin in 2015–2016 were even lower (Figure 2B)
- Antimicrobial non-susceptibility of PCV13-type *S. pneumoniae* pneumonia decreased from 6.7% to 0% for penicillin and from 11.9% to 1.8% for ceftriaxone between 2009–2010 and 2015–2016 in France, Germany, and the UK (Figure 3)

Figure 2A. Antimicrobial Non-susceptibility in all *S. pneumoniae* Pneumonia in Adults ≥50 Years over Time
Ireland, France, Germany, and U.K. Combined

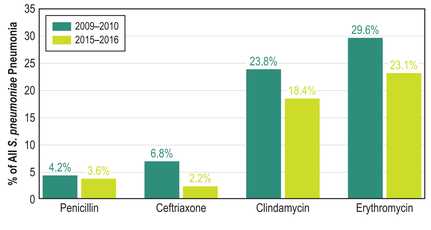
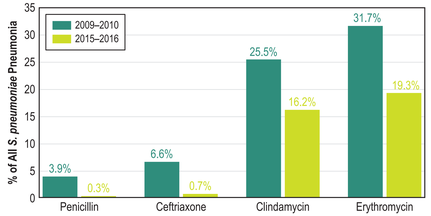


Figure 2B. Antimicrobial Non-susceptibility in *S. pneumoniae* Pneumonia excluding those from Ireland observed in Adults ≥50 Years over Time
France, Germany, and U.K. Combined

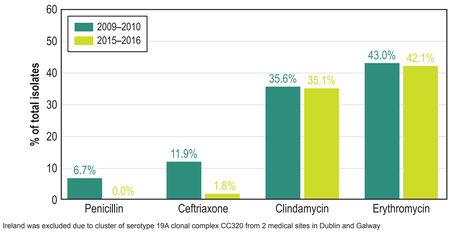


Ireland was excluded due to cluster of serotype 19A clonal complex CC320 from 2 medical sites in Dublin and Galway

Conclusions

- PCV13 paediatric immunization programs in Ireland, France, Germany, and the United Kingdom were associated with substantial declines in the proportion of PCV13-type *S. pneumoniae* in adults, likely resulting from indirect protection caused by reduced transmission from vaccinated children to unvaccinated adults
- By 2015–2016, PCV13-type still accounted for 21.2% of *S. pneumoniae* pneumonia cases, mostly due to serotype19A
  - These data suggest that direct adult PCV13 immunization may have an additional public health benefit beyond that of PCV13 paediatric immunization
- A clonal dissemination of 19A isolates belonging to ST320 and exhibiting a penicillin resistance phenotype was detected in 2 medical sites in Ireland
  - The emergence of a resistant 19A clone in Ireland deserves additional analysis in the context of Ireland's paediatric immunization program
- Routine paediatric use of PCV13 vaccination may have also contributed to a reduced antimicrobial nonsusceptibility in pneumococcal pneumonia in France, Germany, and the United Kingdom, especially for penicillin and ceftriaxone
- These findings appear to have been driven by reductions of both the distribution of PCV13-type disease and reductions in PCV-13-type antimicrobial non-susceptibility

Figure 3. Antimicrobial Non-susceptibility in PCV13-type *S. pneumoniae* Pneumonia in Adults ≥50 Years over Time
*Ireland, France, Germany, and U.K. Combined**



Ireland was excluded due to cluster of serotype 19A clonal complex CC320 from 2 medical sites in Dublin and Galway

Acknowledgements

Pfizer sponsored this poster. JAS and REI are Pfizer employees and may own company stock.

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